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UNITED STATES DEPARTMENT OF COMMERCE

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August 14, 2000

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APPLICATION NUMBER: *60/182,412*
FILING DATE: *February 14, 2000*

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Certifying Officer

02-15-00 A PTO

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Docket Number: BEAU 4A PR

jc688 U.S. PTO 02/14/00

jc710 U.S. PTO 60/182412 02/14/00

PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
André Robert Benoît	Beaulieu Paquin Larivière	Cap-Rouge (Québec), Canada St-Jean-Chrysostome (Québec), Canada St-Nicholas (Québec), Canada

☐ *Additional inventors are being named on page 2 attached hereto*

TITLE OF THE INVENTION (280 characters max)

WOUND HEALING FORMULATIONS CONTAINING HUMAN PLASMA FIBRONECTIN

CORRESPONDENCE ADDRESS

Direct all correspondence to.

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification — *Number of Pages* 112

☒ Drawing(s) — *Number of Sheets* 1, see p.2

☒ Other (specify) claims -- 1 sheet

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

		FILING FEE AMOUNT ($)
☒ A check or money order is enclosed to cover the filing fees		
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:	15-0699	$150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are. _____

Respectfully submitted,

SIGNATURE Manette Dennis DATE February 14, 2000

TYPED or PRINTED NAME Manette Dennis REGISTRATION NO. *(if appropriate)* 30,623

TELEPHONE 212-826-6565

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

P19LARGE/REV04

Docket Number:	BEAU 4A PRV

PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (city and either State or Foreign Country)
Note: There are drawings interspersed with text in the specification. These drawings were not counted separately.		

Certificate of Mailing by Express Mail

I certify that this application and enclosed fee is being deposited on February 14, 2000 with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Signature of Person Mailing Correspondence

Manette Dennis

Typed or Printed Name of Person Mailing Correspondence

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

Efficacy of a new pharmaceutical formulation comprising calcium-alginate and fibronectin (DermaLink delivery system) in stimulating wound healing in the rabbit ear acute wound model. Benoît Larivière, Robert Paquin, Sylvain Picard and André Beaulieu. Dermacor, Sainte-Foy, Québec, Canada

A number of studies have demonstrated the efficacy of solutions of fibronectin in stimulating wound healing in various animal models. These include skin rat models and rabbit corneal ulcer models. As solutions of fibronectin may not be appropriate for clinical use, Dermacor has developed a new pharmaceutical formulation in which fibronectin is embodied in calcium-alginate (DermaLink delivery system). Studies on the efficacy of DermaLink delivery system in stimulating wound healing were conducted using the rabbit ear acute dermal wound model. In this model, dermal wounds are created on the rabbit ear of New Zealand white rabbits with a 6 mm punch biopsy. The dermis is removed and bare cartilage is exposed. Four wounds may be created per ear. As cartilage is non-vascularized, new granulation tissue formation occurs only at the periphery of the wound. One ear is used for the active treatment sites and the other ear is used for the control sites. In this case, DermaLink delivery system was applied on the wounds of one ear and calcium-alginate dressing without fibronectin was applied to wounds on the other ear. Tegaderm™ dressing was next applied to cover the wounds and left in place for a period of 8 days. The studied variables were the Mean Height of new granulation tissue (MH in mm) at the wound periphery and the New Granulation Volume (NGV in mm^3). Results showed a dose response effect of DermaLink delivery system when compared to calcium-alginate alone over doses ranging from 10 to 120 ug of fibronectin delivered per mm^2 of wound surface area. With the doses of 40, 80 and 120 ug/mm^2 of fibronectin, both MH and NGV were significantly higher than the control with P values of <0.0001 in both cases. These findings demonstrate that DermaLink delivery system has the ability to stimulate new granulation tissue in a formulation that is appropriate for clinical use.

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Rapport NAN 23

Study of the effect of fibronectin with the Rabbit Dermal Ulcer Model.

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Claude Laberge

STATEX

1- Studied variables :

MH M (mm) :
NGV :
NGT GAP M :

2- Between 12 and 13 rabbits studied by Fibronectin dose.

3- Six doses studied : 10.3, 20.6, 41.25, 82.5, 123.75 and 165 $\mu g/mm^2$

Statistical methodology :

-Analysis of variance

-Two factors : SITE, 4 levels (a, b, c and d)
TREAT, 2 levels (Placebo, Fibronectin)

-Analyses are performed for each Fibronectin dose

-Rabbits are used as statistical blocks.

Variable MH M,

A) Dose 10.3 $\mu g/mm^2$

Fibronectin effect non significant (p=0.25)

Site effect non significant (p=0.29)

Interaction Site*Fibronectin non significant (p=0.10)

Conclusion :

At this dose (10.3 $\mu g/mm^2$), Fibronectin has no effect on MH M. Furthermore, the absence of effect is true for all sites.

Graphical representations of the fibronectin effect for all sites and rabbits are used to illustrate the results. In these graphs :

-Each line is associated to a rabbit.

-A upward slope indicate that the rabbit has answered positively to fibronectin in comparison with the placebo.

-A significant fibronectin effect will generally be associated to parallel lines with upward slope.

-A significant site effect will be associated to group of lines at different levels from one site plot to the other

-A significant interaction site*fibronectin is associated to different line slopes from one site plot to the other.

Figure 1 shows :

-Rabbits show different effects of fibronectin.

-Groups of lines are at similar levels from site to site.

-No fibronectin and site effects.

Figure 1. Graphical representation of the fibronectin (10.3 μg/mm^2) effect on MH M for all sites and rabbits.

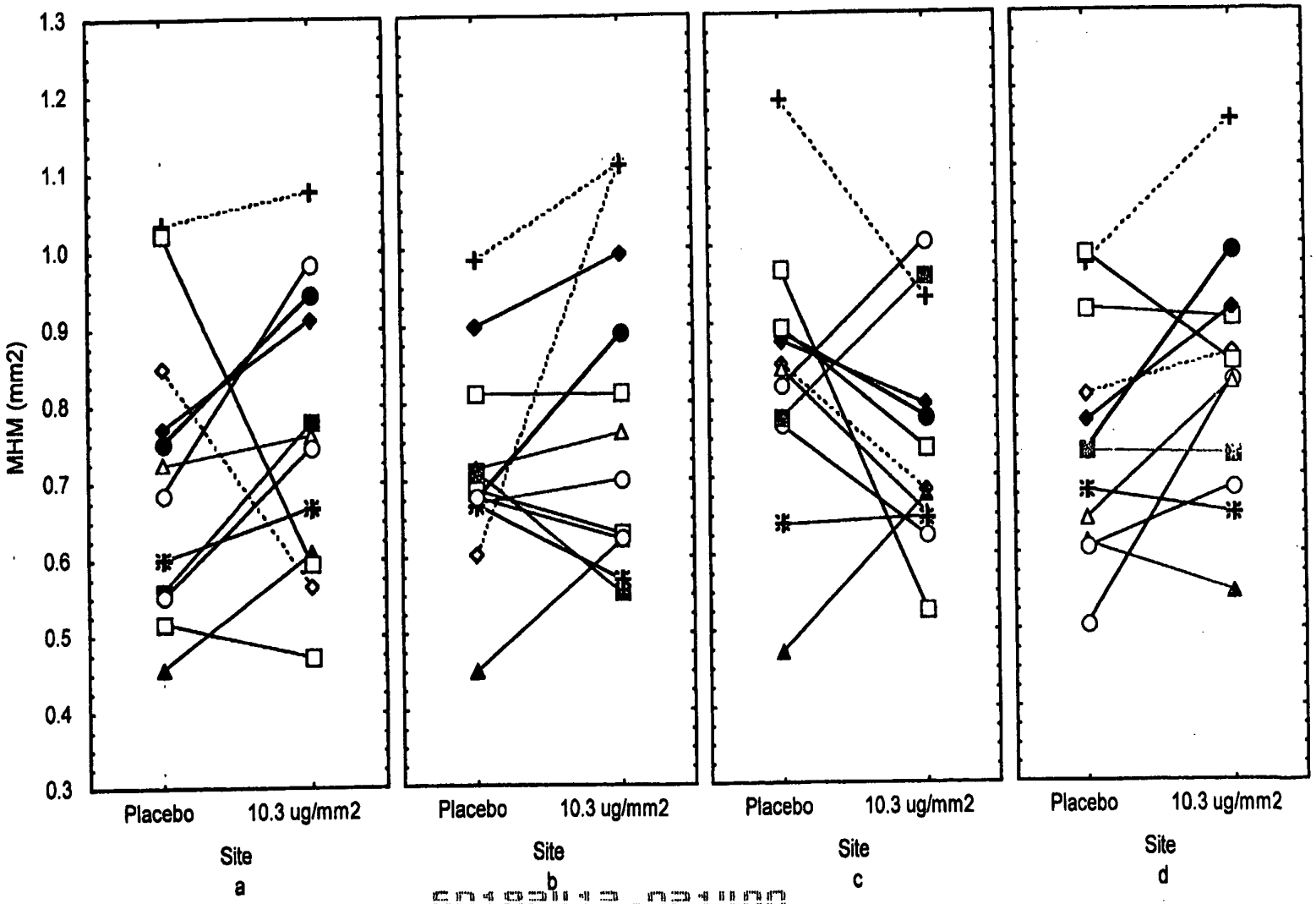


Figure 2 shows :

-Several rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on site d. Nevertheless, the non significant interaction site*fibronectin indicates that, statistically, all sites respond the same way to a 20.6 $\mu g/mm^2$ dose of fibronectin.

Variable MH M,

C) Dose 41.25 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.44$)

Interaction Site*Fibronectin non significant ($p=0.56$)

Conclusion :

At this dose (41.25 $\mu g/mm^2$), Fibronectin has an effect on MH M. Furthermore, the absence of interaction implies that the conclusion can be generalized to all sites.

Figure 3. Graphical representation of the fibronectin (41.25 µg/mm^2) effect on MH M for all sites and rabbits.

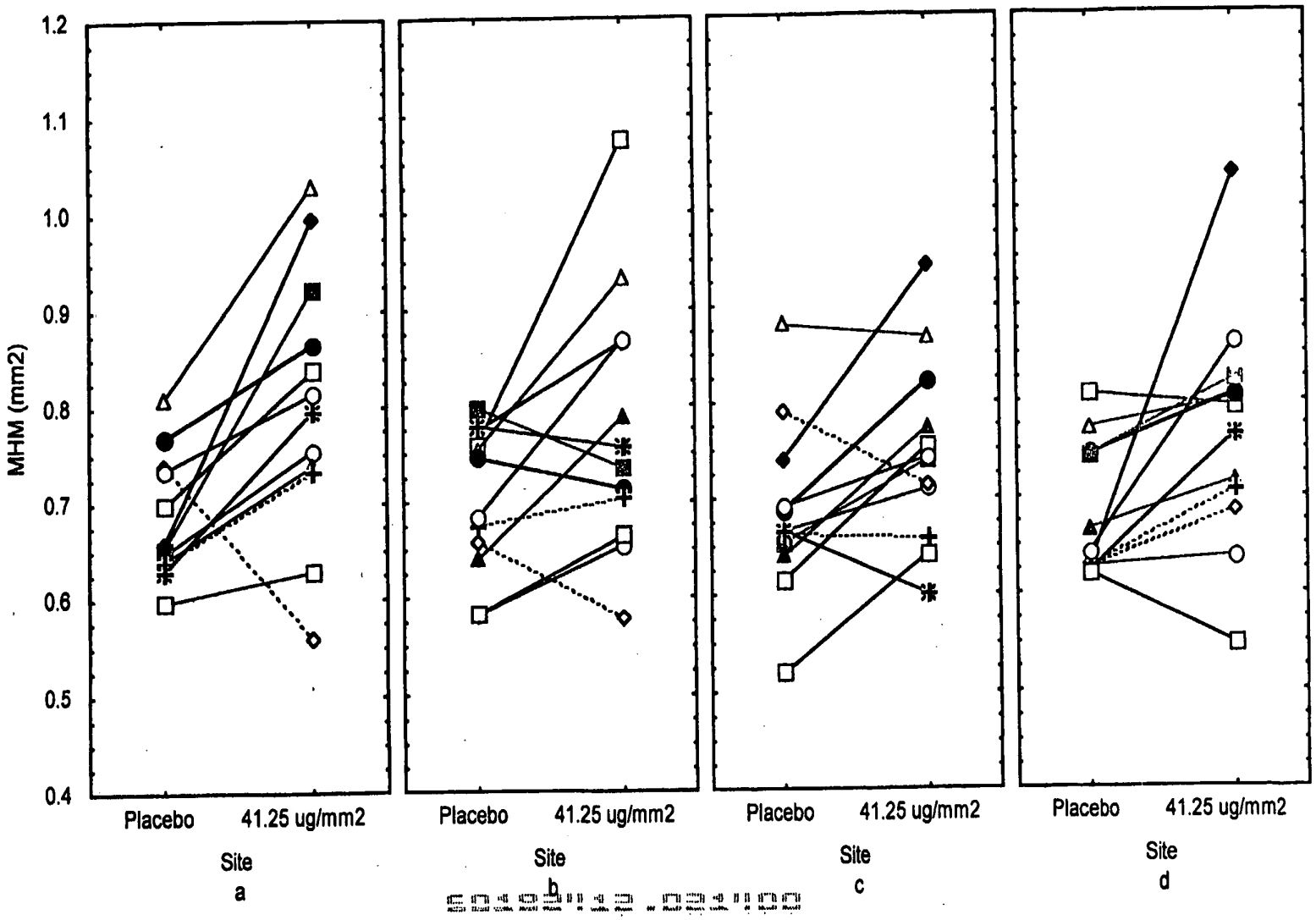


Figure 3 shows :

-Several rabbits show a positive effect of fibronectin.
Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on sites a and d.
Nevertheless, the non significant interaction site*fibronectin indicates that, statistically, all sites respond the same way to a 41,25 $\mu g/mm^2$ dose of fibronectin.

Variable MH M,

D) Dose 82.5 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.09$)

Interaction Site*Fibronectin is significant ($p=0.002$)

Conclusion :

At this dose (82.5 $\mu g/mm^2$), Fibronectin has an effect on MH M. The presence of a significant interaction implies that the conclusion may differ for some sites.

Figure 4. Graphical representation of the fibronectin (82.5 μg/mm^2) effect on MH M for all sites and rabbits.

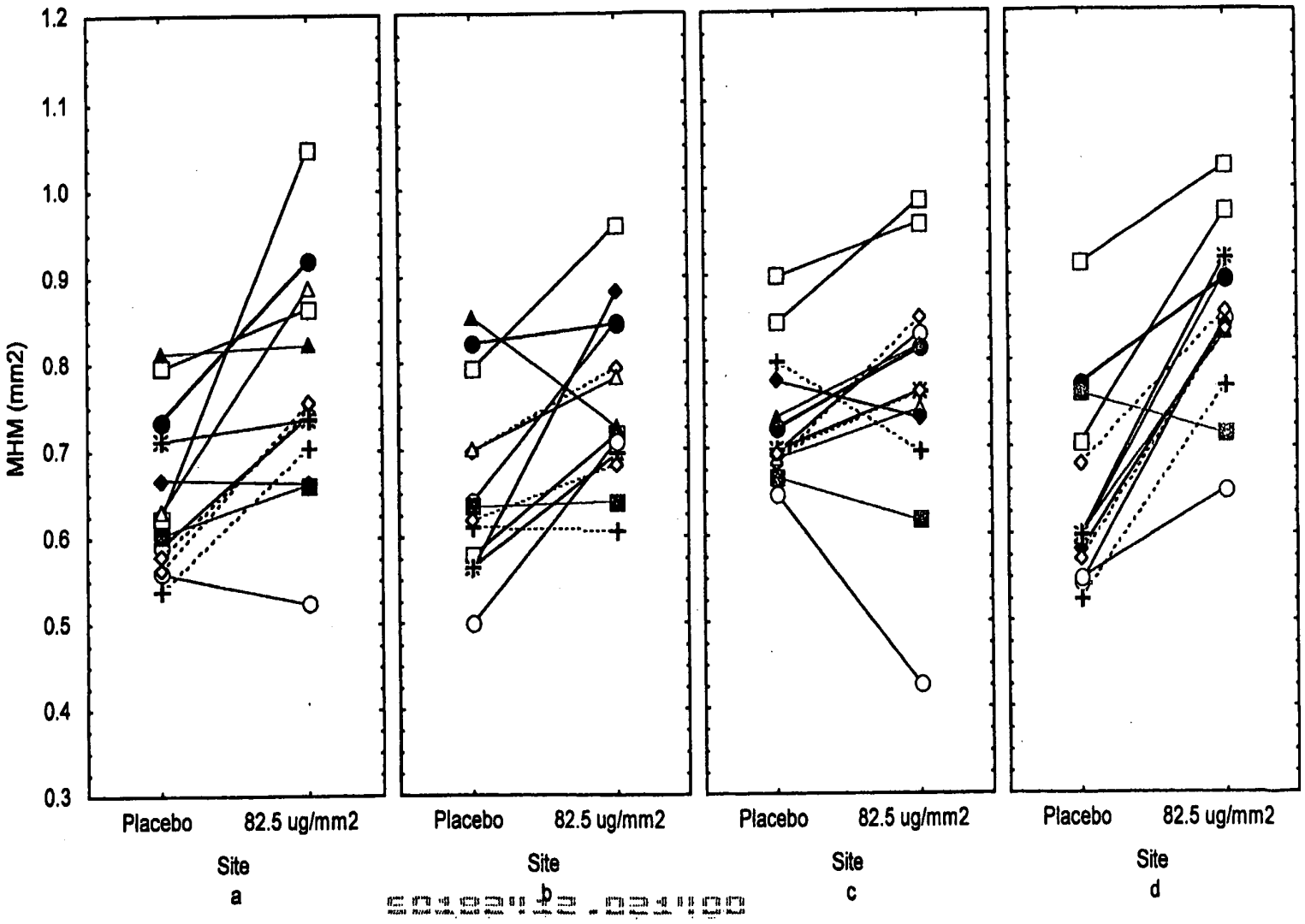
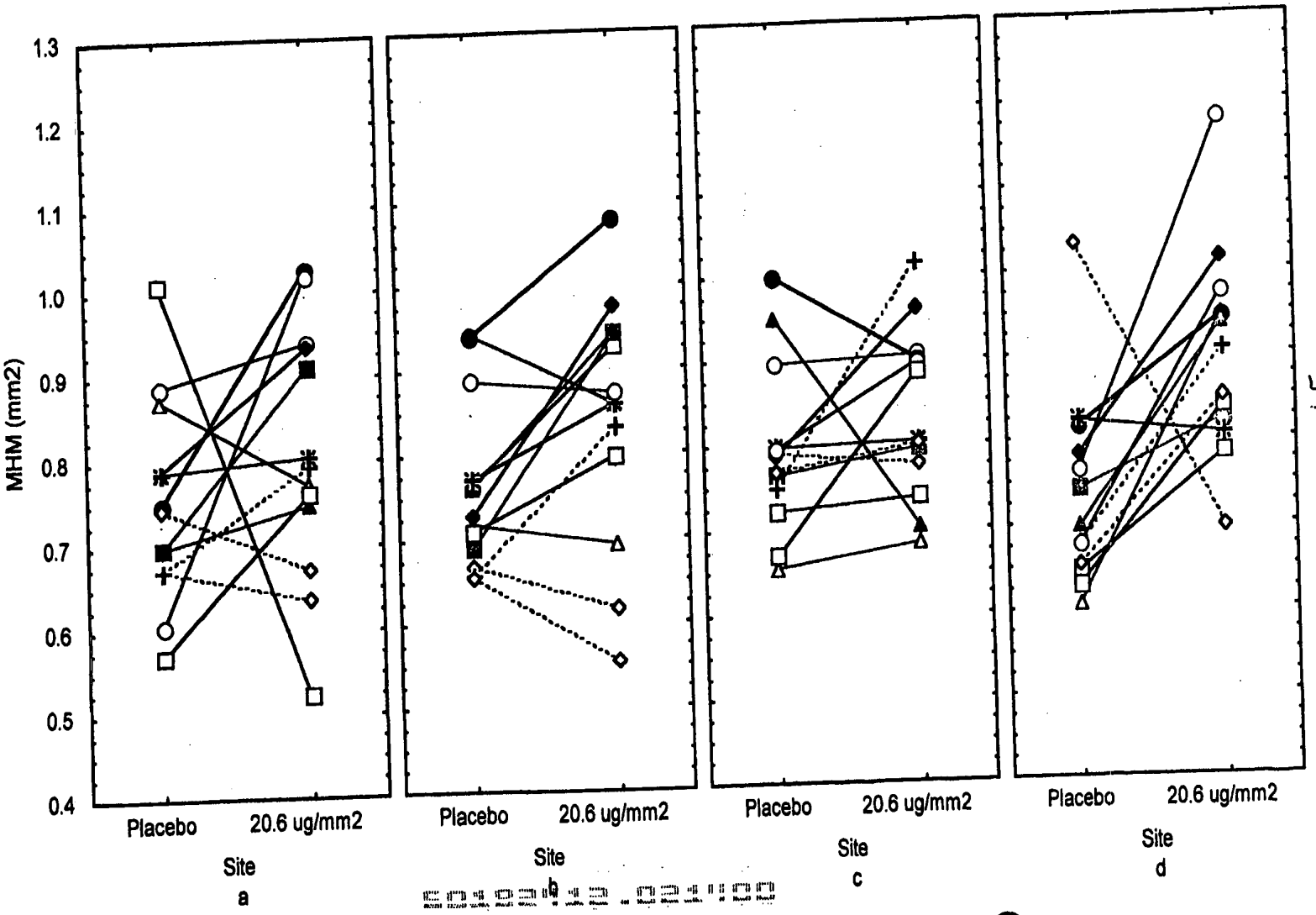


Figure 2. Graphical representation of the fibronectin (20.6 µg/mm^2) effect on MH M for all sites and rabbits.



Variable MH M,

B) Dose 20.6 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.57$)

Interaction Site*Fibronectin non significant ($p=0.16$)

Conclusion :

At this dose (20.6 $\mu g/mm^2$), Fibronectin has an effect on MH M. Furthermore, the absence of interaction implies that the conclusion can be generalized to all sites.

Figure 4 shows :

-Several rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on site d which could explain the significant site*fibronectin interaction.

Variable MH M,

E) Dose 123.75 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.4$)

Interaction Site*Fibronectin is significant ($p=0.04$)

Conclusion :

At this dose (123.75 $\mu g/mm^2$), Fibronectin has an effect on MH M. The presence of a significant interaction implies that the conclusion may differ for some sites.

Figure 5. Graphical representation of the fibronectin (123.75 μg/mm^2) effect on MH M for all sites and rabbits.

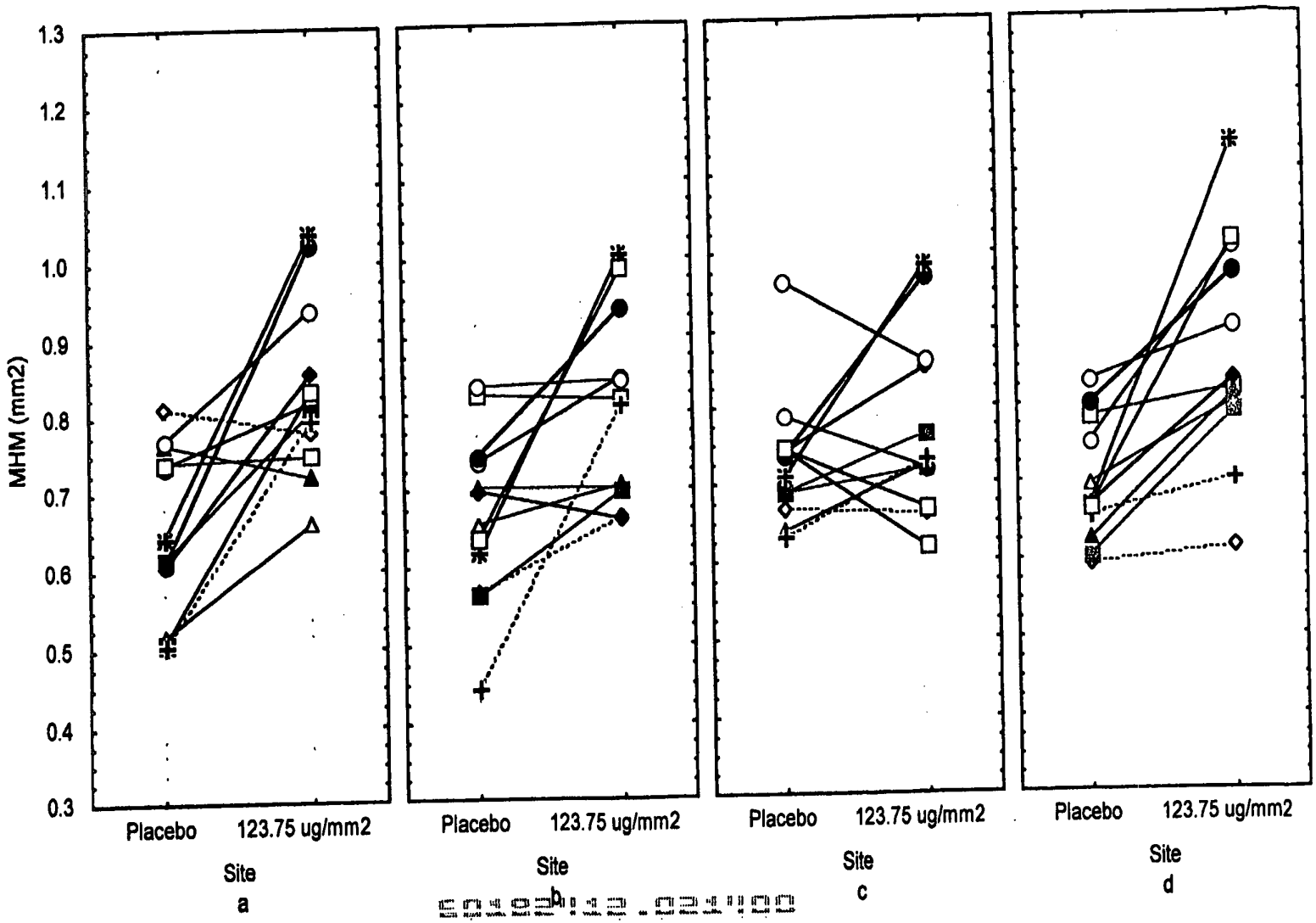


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Figure 5 shows :

-A large majority of rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on sites a and d which could explain the significant site*fibronectin interaction.

Variable MH M,

F) Dose 165 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.29$)

Interaction Site*Fibronectin is significant ($p=0.0054$)

Conclusion :

At this dose (165 $\mu g/mm^2$), Fibronectin has an effect on MH M. The presence of a significant interaction implies that the conclusion may differ for some sites.

Figure 6. Graphical representation of the fibronectin (165 μg/mm^2) effect on MH M for all sites and rabbits.

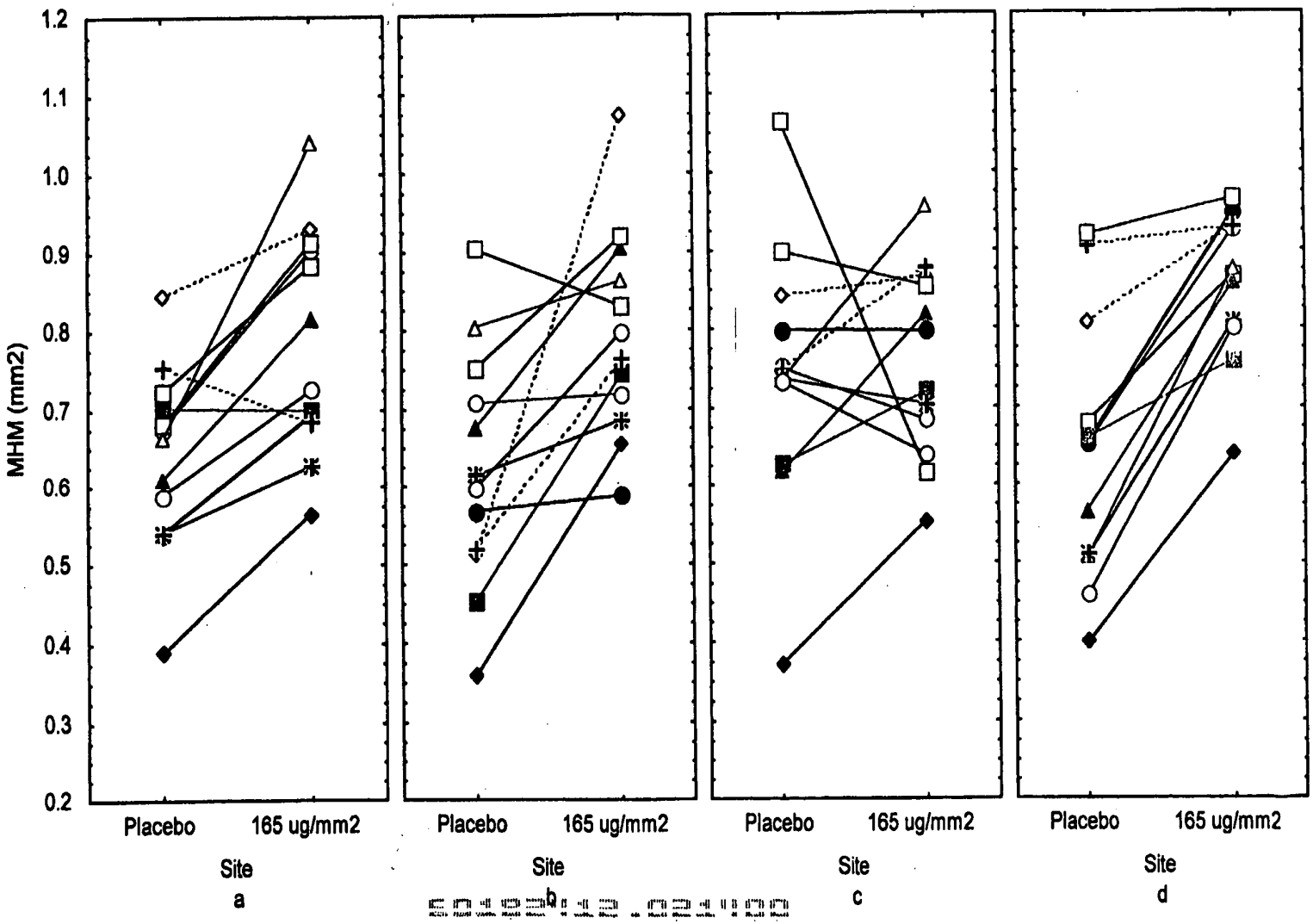


Figure 6 shows :

-A large majority of rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears less important on site c which could explain the significant interaction site*fibronectin.

Summary of the ANOVA results for variable MH M

	n rabbits	Nb ulcers	Site	Treatment	Localisation*Site
10.3 µg/mm^2	12	96	p=0.294	p=0.248	p=0.098
20.6 µg/mm^2	13	104	p=0.571	**p<0.0001**	p=0.159
41.25 µg/mm^2	12	96	p=0.444	**p<0.0001**	p=0.556
82.5 µg/mm^2	13	104	p=0.089	**p<0.0001**	**p=0.0021**
123.75 µg/mm^2	12	96	p=0.397	**p<0.0001**	**p=0.0398**
165 µg/mm^2	12	96	p=0.331	**p<0.0001**	**p=0.0052**

Summary of the ANOVA results for variable MH M : by site and dose to illustrate the significant interactions

DOSE	Site a	Site b	Site c	Site d
10.3 $\mu g/mm^2$	0.45	0.22	0.19	0.073
20.6 $\mu g/mm^2$	0.35	**0.036**	0.30	**0.008**
41.25 $\mu g/mm^2$	**0.0075**	**0.049**	**0.033**	**0.032**
82.5 $\mu g/mm^2$	**0.0034**	**0.0083**	0.27	**<0.0001**
123.75 $\mu g/mm^2$	**0.0021**	**0.010**	0.27	**0.0011**
165 $\mu g/mm^2$	**0.0010**	**0.0051**	0.80	**<0.0001**

Variable NGV,

A) Dose 10.3 $\mu g/mm^2$

Fibronectin effect non significant (p=0.61)

Site effect non significant (p=0.55)

Interaction Site*Fibronectin non significant (p=0.24)

Conclusion :

At this dose (10.3 $\mu g/mm^2$), Fibronectin has no effect on NGV. Furthermore, the absence of effect is true for all sites.

Figure 7. Graphical representation of the fibronectin (10.3 μg/mm^2) effect on NGV for all sites and rabbits.

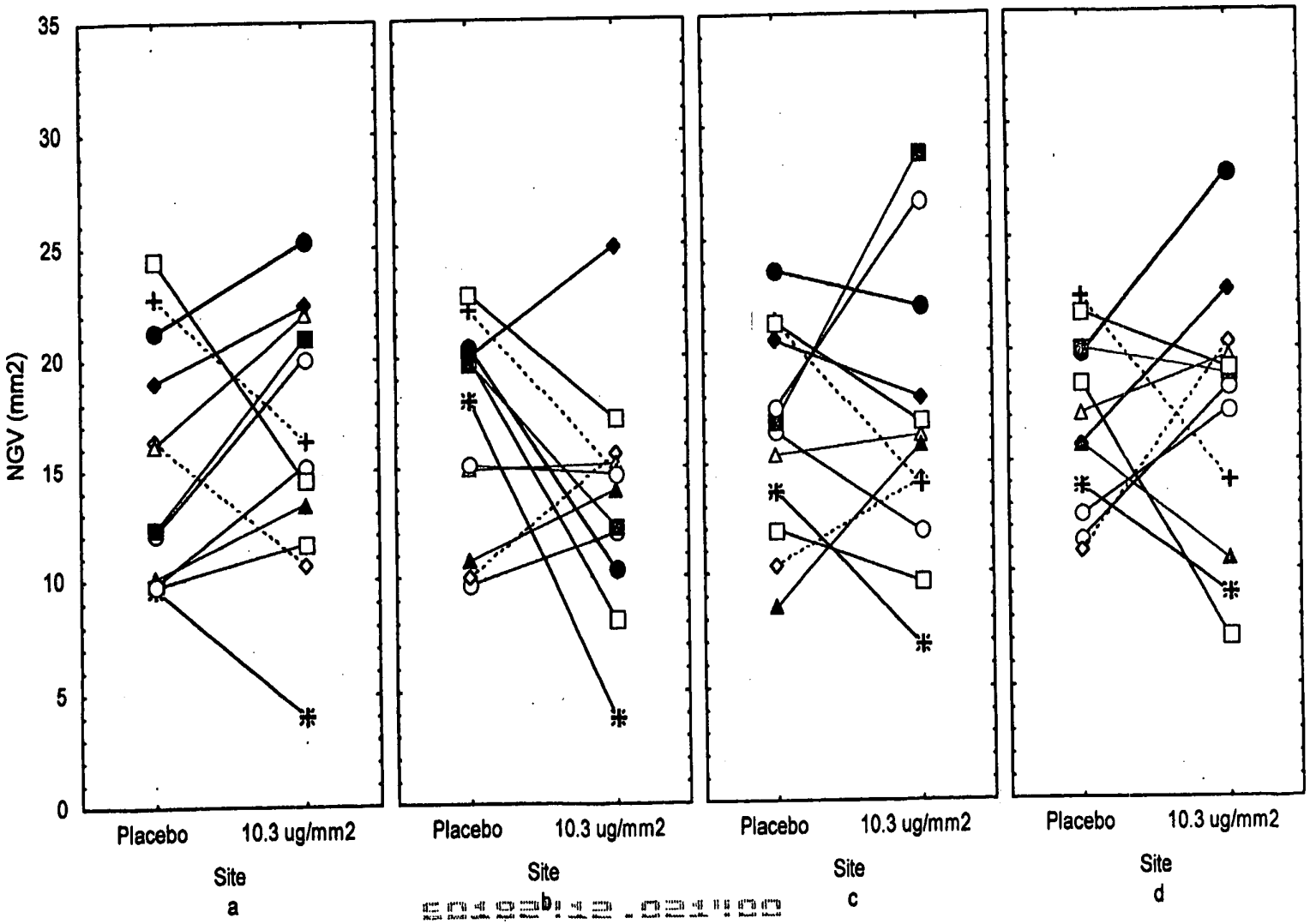


Figure 7 shows :

-Rabbits show different effects of fibronectin.

-Groups of lines are at similar levels from site to site.

-No fibronectin and site effects.

Variable NGV,

B) Dose 20.6 $\mu g/mm^2$

Fibronectin effect non significant ($p=0.28$)

Site effect non significant ($p=0.60$)

Interaction Site*Fibronectin non significant ($p=0.064$)

Conclusion :

At this dose (20.6 $\mu g/mm^2$), Fibronectin doesn't have an effect on NGV. Furthermore, the absence of interaction implies that the conclusion can be generalized to all sites.

Figure 8. Graphical representation of the fibronectin (20.6 μg/mm^2) effect on NGV for all sites and rabbits.

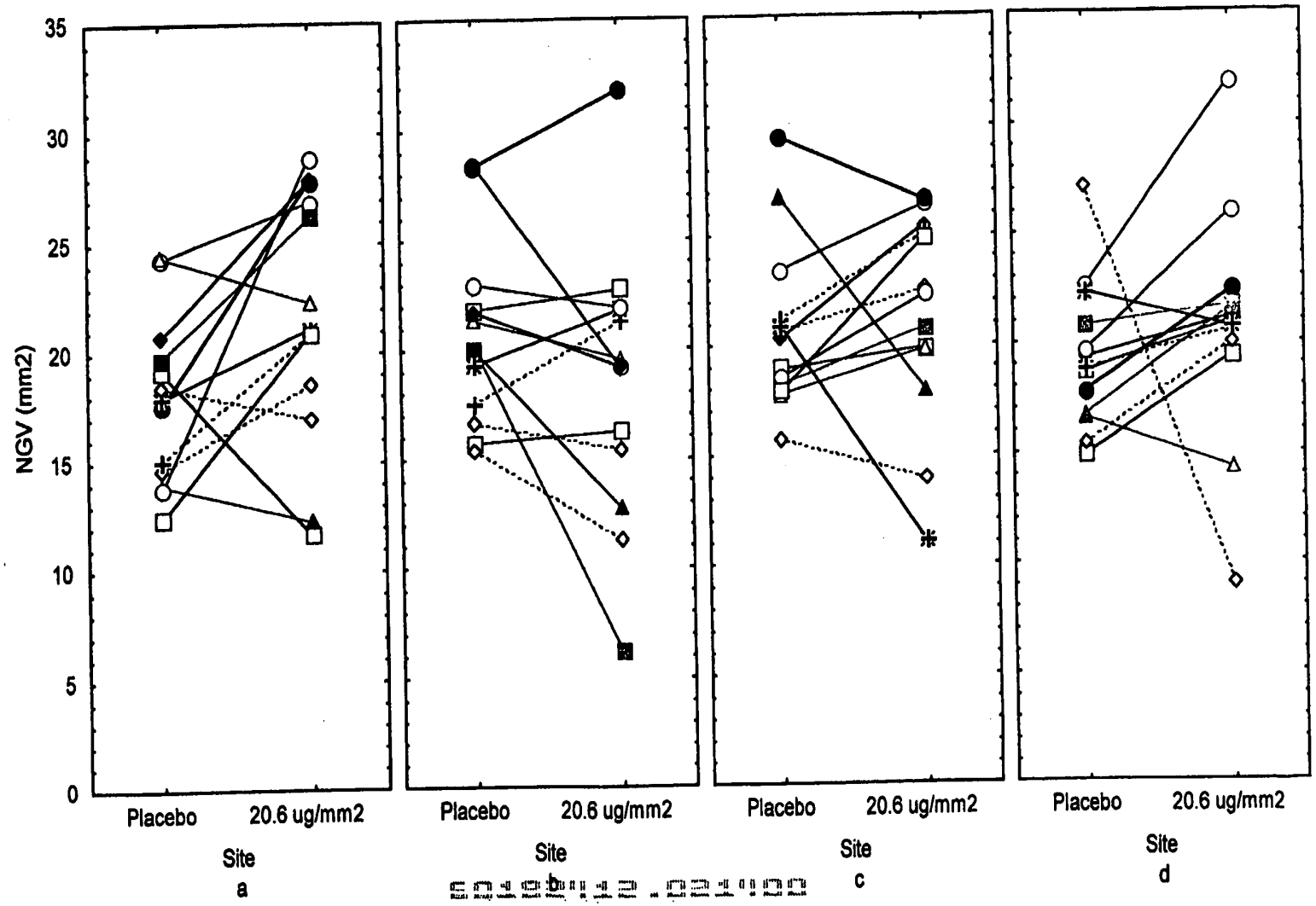


Figure 8 shows :

-Rabbits show different effects of fibronectin.

-Groups of lines are at similar levels from site to site.

-No fibronectin and site effects.

Variable NGV,

C) Dose 41.25 $\mu g/mm^2$

Fibronectin effect is significant (p=0.0008)

Site effect non significant (p=0.88)

Interaction Site*Fibronectin non significant (p=0.46)

Conclusion :

At this dose (41.25 $\mu g/mm^2$), Fibronectin has an effect on NGV. Furthermore, the absence of interaction implies that the conclusion can be generalized to all sites.

Figure 9. Graphical representation of the fibronectin (41.25 µg/mm^2) effect on NGV for all sites and rabbits.

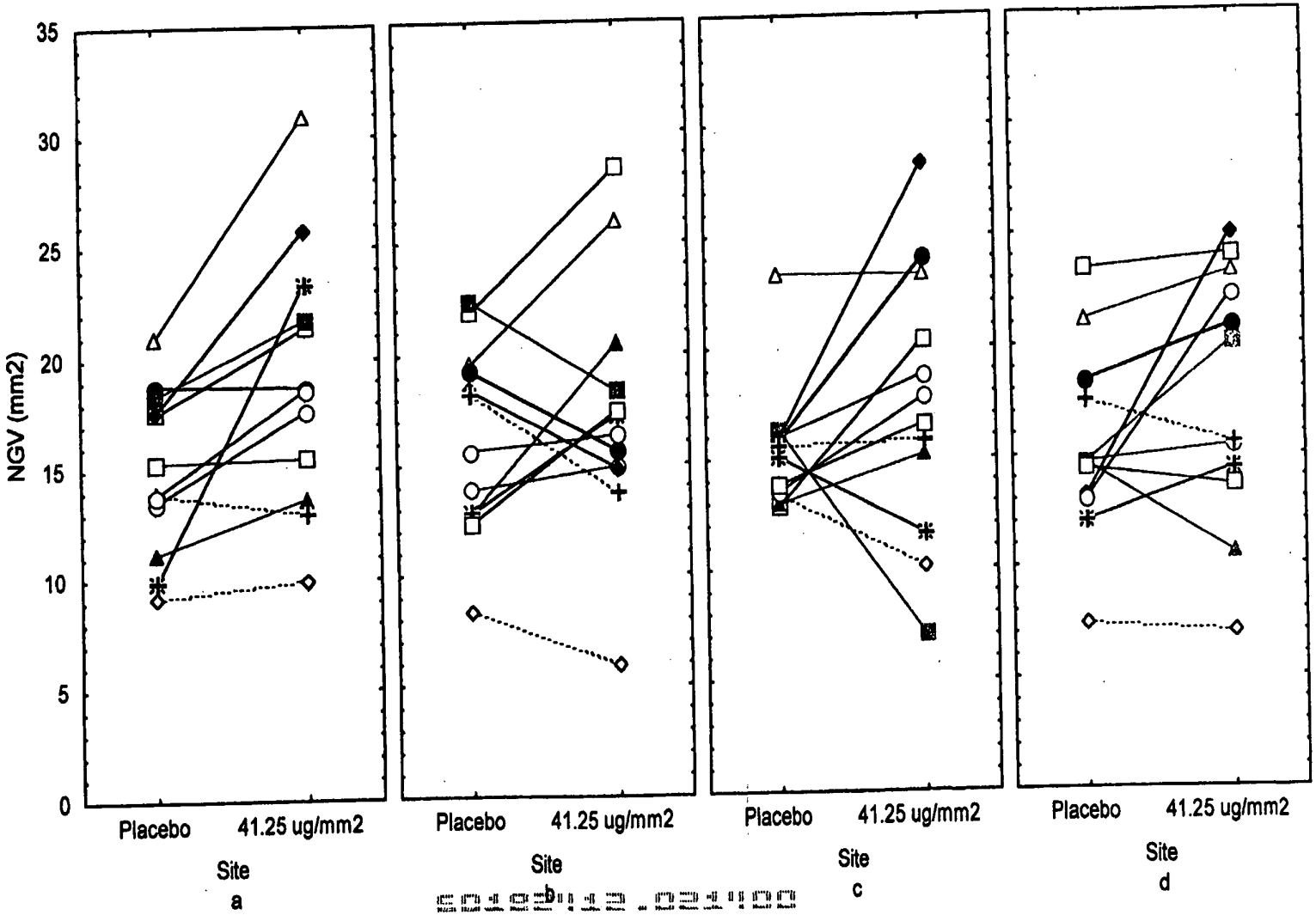


Figure 9 shows :

-Several rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears similar on all sites which explains the non significant site*fibronectin interaction and indicates that, statistically, all sites respond the same way to a 41.25 $\mu g/mm^2$ dose of fibronectin.

Variable NGV,

D) Dose 82.5 $\mu g/mm^2$

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.19$)

Interaction Site*Fibronectin is significant ($p=0.0015$)

Conclusion :

At this dose (82.5 $\mu g/mm^2$), Fibronectin has an effect on MH M. The presence of a significant interaction implies that the conclusion may differ for some sites.

Figure 10. Graphical representation of the fibronectin (82.5 μg/mm^2) effect on NGV for all sites and rabbits.

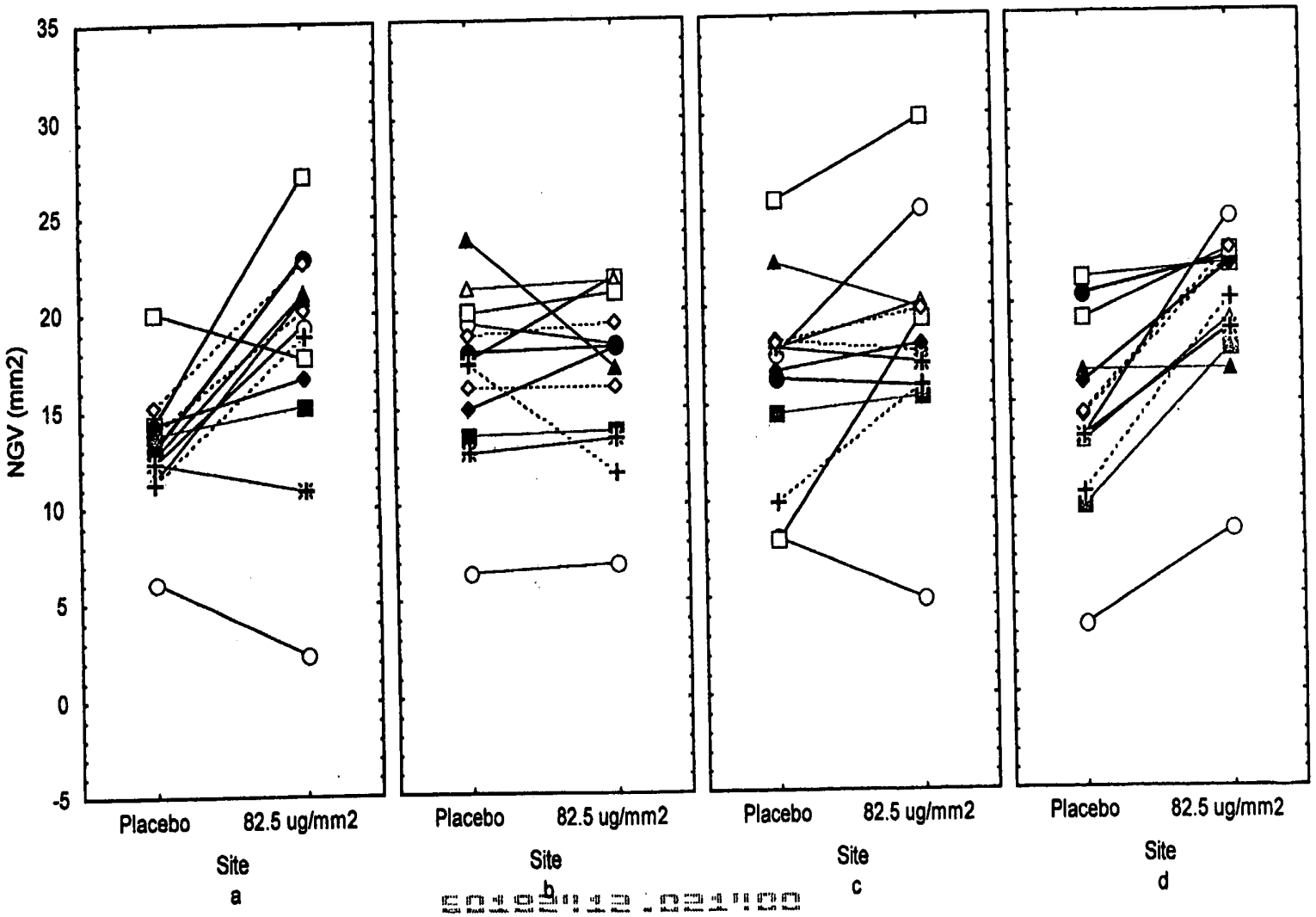


Figure 10 shows :

-Several rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on sites a and d which could explain the significant interaction site*fibronectin.

Variable NGV,

E) Dose 123.75 µg/mm^2

Fibronectin effect is significant ($p<0.0001$)

Site effect non significant ($p=0.72$)

Interaction Site*Fibronectin is significant ($p=0.0039$)

Conclusion :

At this dose (123.75 µg/mm^2), Fibronectin has an effect on MH M. The presence of a significant interaction implies that the conclusion may differ for some sites.

Figure 11. Graphical representation of the fibronectin (123.75 µg/mm^2) effect on NGV for all sites and rabbits.

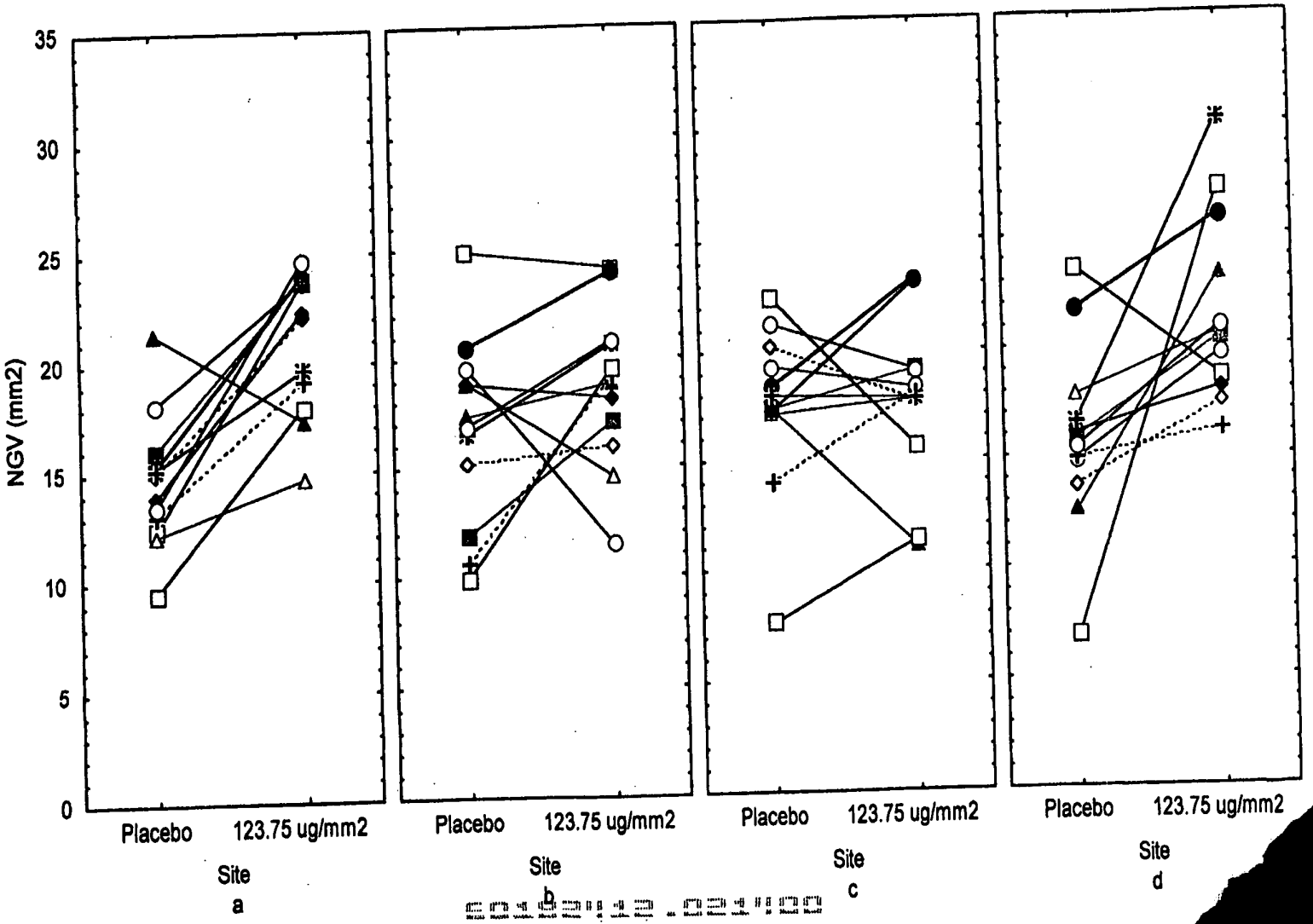


Figure 11 shows :

-Several rabbits show a positive effect of fibronectin. Significant fibronectin effect even though some rabbits show a negative fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect appears more important on sites a and d which could explain the significant interaction site*fibronectin.

Variable NGV,

F) Dose 165 $\mu g/mm^2$

Fibronectin effect non significant ($p=0.124$)

Site effect non significant ($p=0.94$)

Interaction Site*Fibronectin non significant ($p=0.059$)

Conclusion :

At this dose (165 $\mu g/mm^2$), Fibronectin doesn't have an effect on NGV. Furthermore, the absence of interaction implies that the conclusion can be generalized to all sites.

Figure 12. Graphical representation of the fibronectin (165 µg/mm^2) effect on NGV for all sites and rabbits.

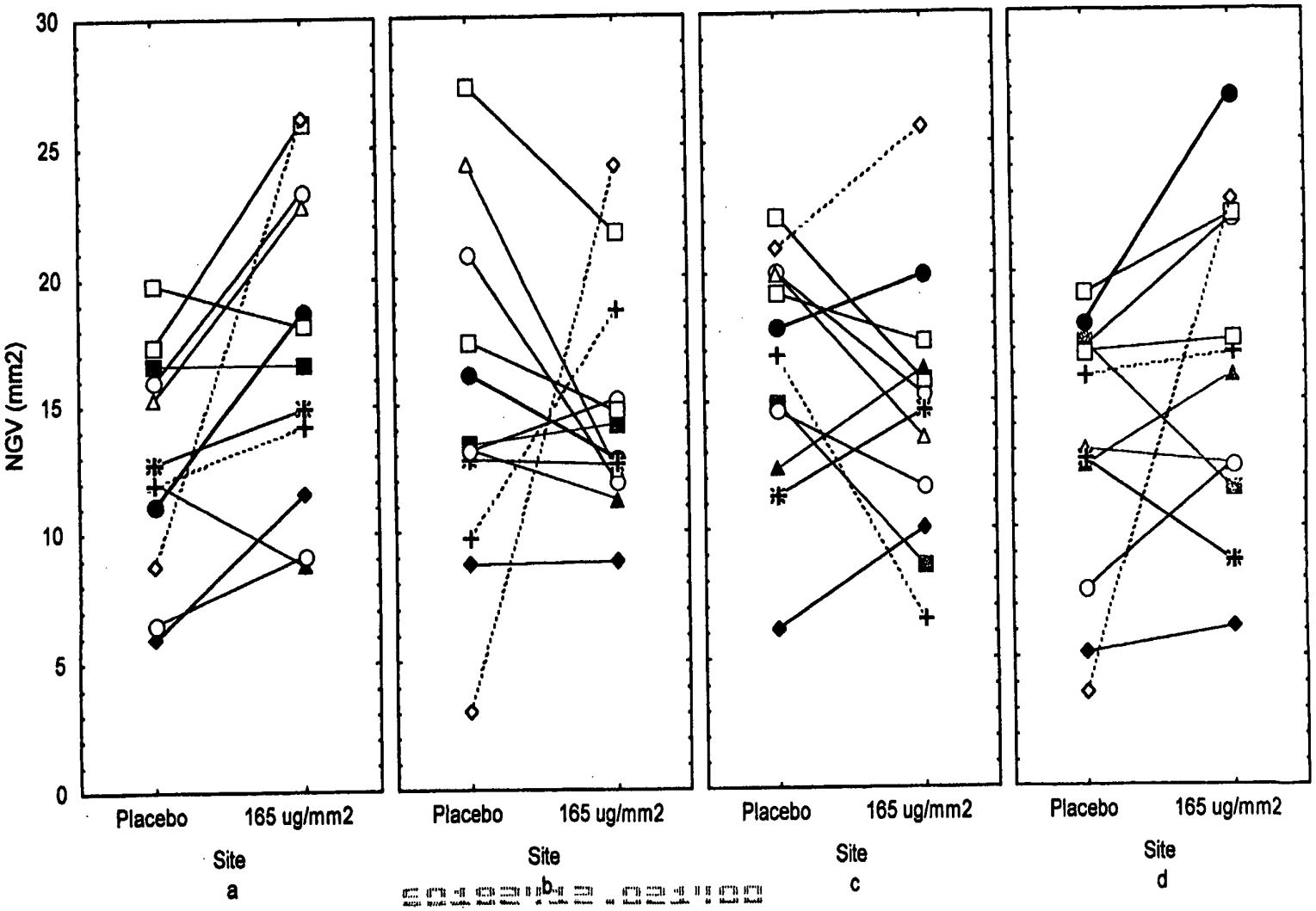


Figure 12 shows :

-Several rabbits show a positive effect of fibronectin but several rabbits also show a negative effect of fibronectin (particularly for sites b and c). The different effects on different rabbits explain the non significant fibronectin effect.

-Groups of lines are at similar levels from site to site.

-The fibronectin effect has disappeared at a 165 $\mu g/mm^2$ dose of fibronectin.

Summary of the ANOVA results for variable NGV

	n rabbits	Nb ulcers	Site	Treatment	Localisation*Site
10.3 µg/mm^2	12	96	p=0.550	p=0.612	p=0.239
20.6 µg/mm^2	13	104	p=0.595	p=0.282	p=0.064
41.25 µg/mm^2	12	96	p=0.881	**p=0.0008**	p=0.459
82.5 µg/mm^2	13	104	p=0.189	**p<0.0001**	**p=0.0015**
123.75 µg/mm^2	12	96	p=0.721	**p<0.0001**	**p=0.0039**
165 µg/mm^2	12	96	p=0.936	p=0.104	**p=0.0434**

Summary of the ANOVA results for variable NGV :
by site and dose to illustrate the significant interactions

DOSE	Site a	Site b	Site c	Site d
10.3 $\mu g/mm^2$	0.57	0.11	0.89	0.86
20.6 $\mu g/mm^2$	0.039	0.13	0.71	0.45
41.25 $\mu g/mm^2$	**0.0072**	0.43	0.26	0.11
82.5 $\mu g/mm^2$	**0.0047**	0.81	0.088	**<0.0001**
123.75 $\mu g/mm^2$	**0.0002**	0.22	0.91	**0.011**
165 $\mu g/mm^2$	**0.0144**	0.95	0.26	0.13

Dose Response

In order to show the dose response relation, two graphs are presented for each variable (MH M and NGV).

-A bar graph showing the response of both groups (control and fibronectin) for rabbits associated to each fibronectin dose.

-A scatterplot showing the dose response curve for the difference between the fibronectin and control responses.

Figure 13

Responses for control and fibronectin groups for variable MH M

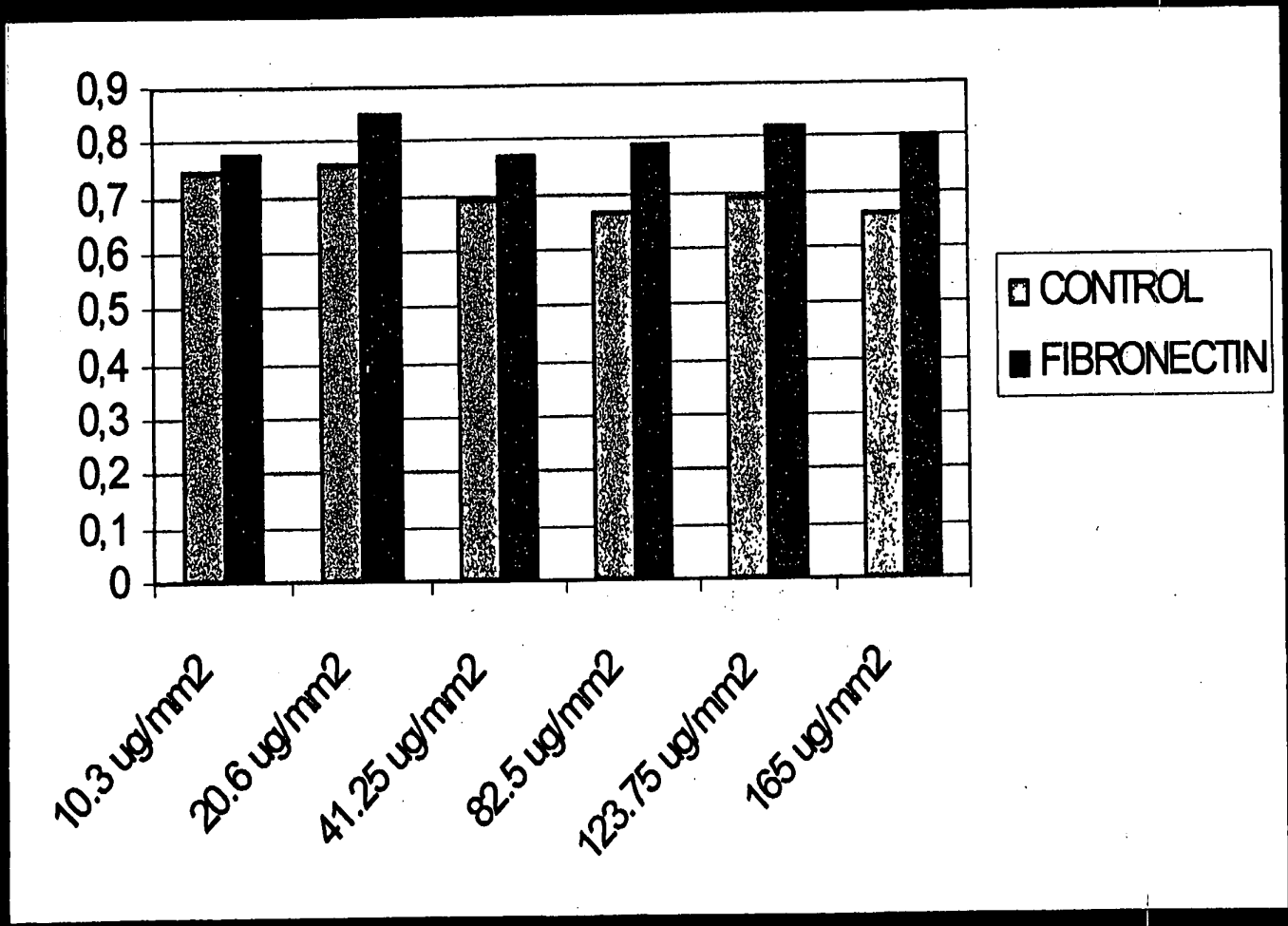


Figure 13 shows that the rabbits used for the 20.6 $\mu g/mm^2$ dose of fibronectin responded very highly to the fibronectin treatment.

However, the higher response for the control ulcers show that this group of rabbits probably had better healing possibilities.

This result explains why the dose response curve should be computed with the difference between the fibronectin and control groups of ulcers.

We can thus say that the dose response curves is adjusted for the rabbit effect.

Figure 14

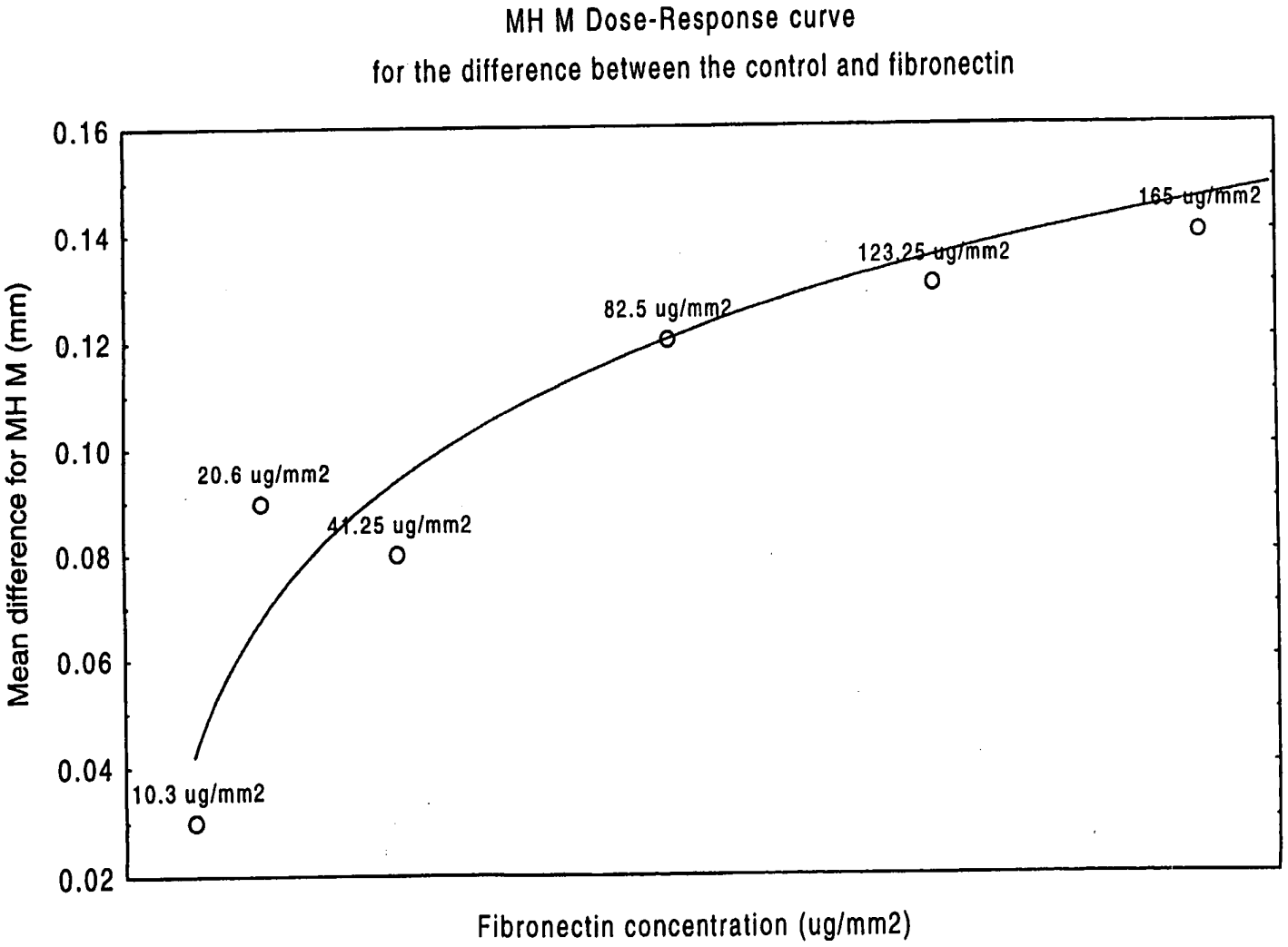

MH M Dose-Response curve
for the difference between the control and fibronectin
Mean difference for MH M (mm)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
10.3 ug/mm2
20.6 ug/mm2
41.25 ug/mm2
82.5 ug/mm2
123.25 ug/mm2
165 ug/mm2
Fibronectin concentration (ug/mm2)

Figure 14 shows an adjusted dose response curve with the response increasing rapidly for small doses and reaching a plateau for the highest concentrations.

The use of an adjusted dose response curve is the most adequate way to represent the response of the rabbits to the treatment because :

-The control groups have different responses showing different intrinsic healing possibilities.
-The use of a paired experiment allow an adjustment for each subject.
-The difference between the control (placebo) and the treatment (fibronectin) is a good estimation of the real effect of the treatment.

Without the use of an adjusted dose response curve, we would conclude that the optimal use of fibronectin is at a 20.6 ug/mm2 dose since higher concentrations show a lower absolute response on MH M. However, the difference between the control and treatment group is not significant at that dose. The adjusted dose response curve indicates that even if the absolute response for 165 ug/mm2 is smaller that the absolute response for 20.6 ug/mm2, the intrinsic characteristics showed by the control groups results show that the absolute response for 165 ug/mm2 would have been higher if similar group of rabbits were used.

Figure 15

Responses for control and fibronectin groups for variable NGV

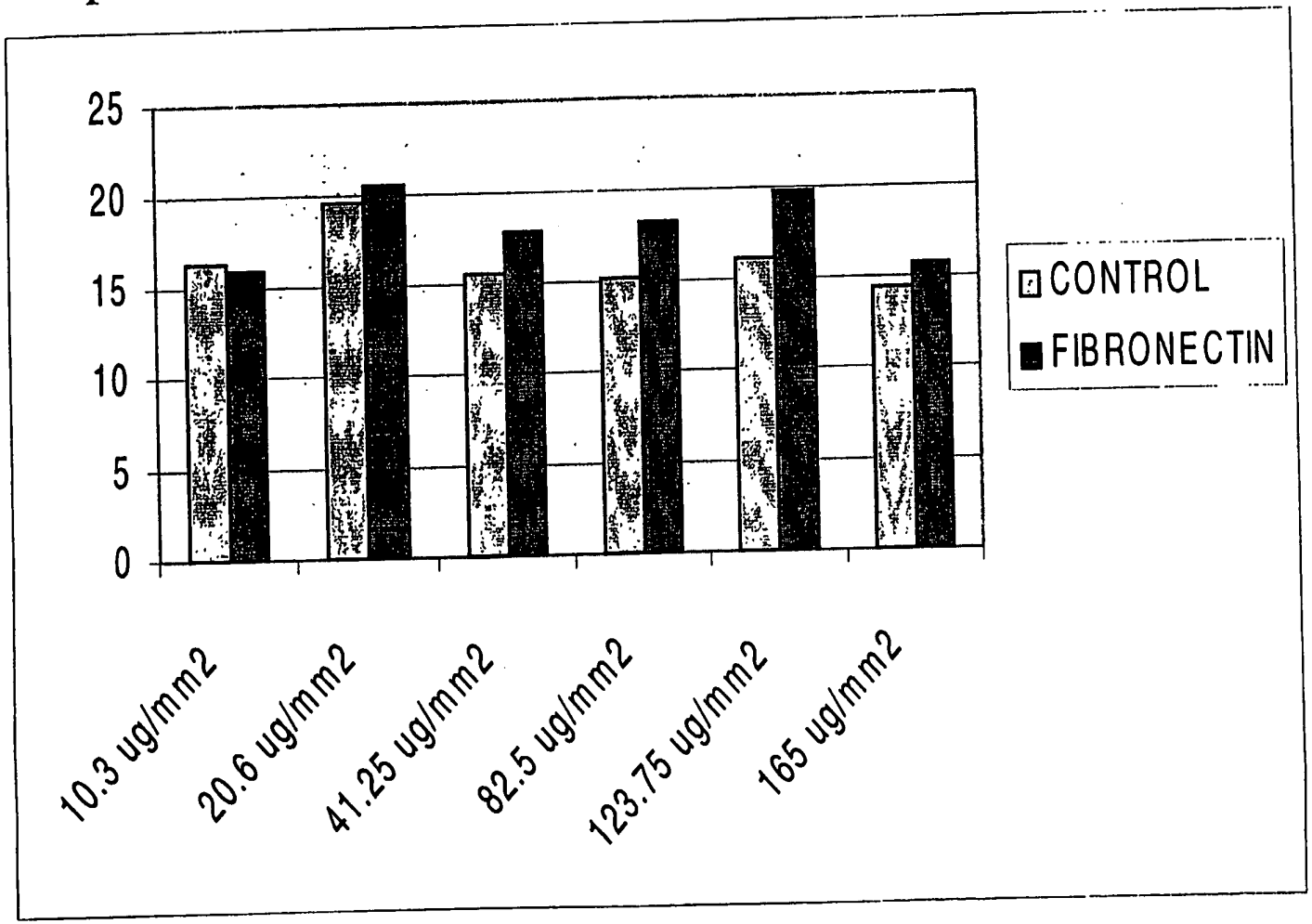


Figure 15 shows that the rabbits used for the 20.6 $\mu g/mm^2$ dose of fibronectin responded very highly to the fibronectin treatment.

However, the higher response for the control ulcers show that this group of rabbits probably had better healing possibilities.

This result explains why the dose response curve should be computed with the difference between the fibronectin and control groups of ulcers.

We can thus say that the dose response curves is adjusted for the rabbit effect.

Figure 16

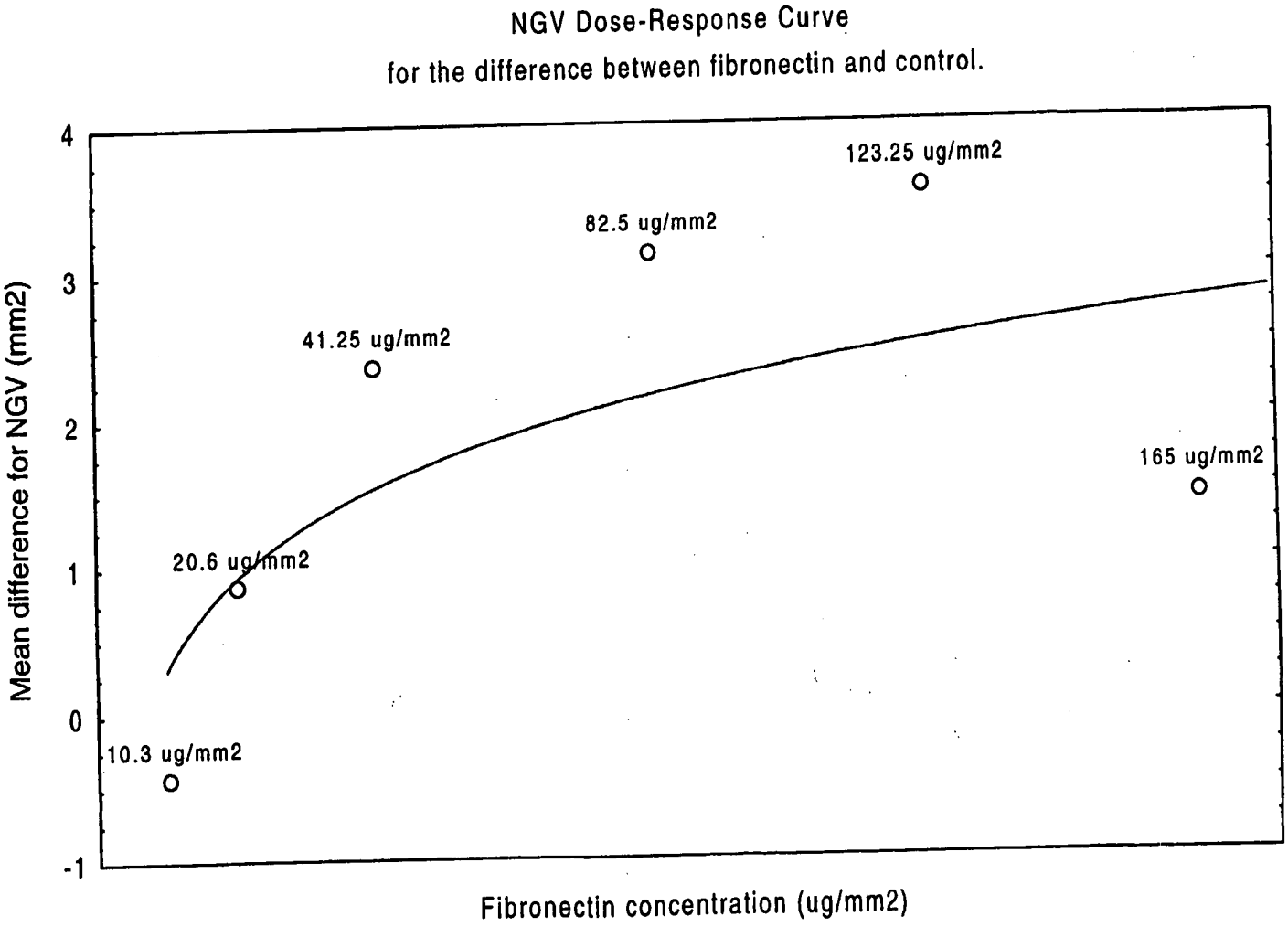
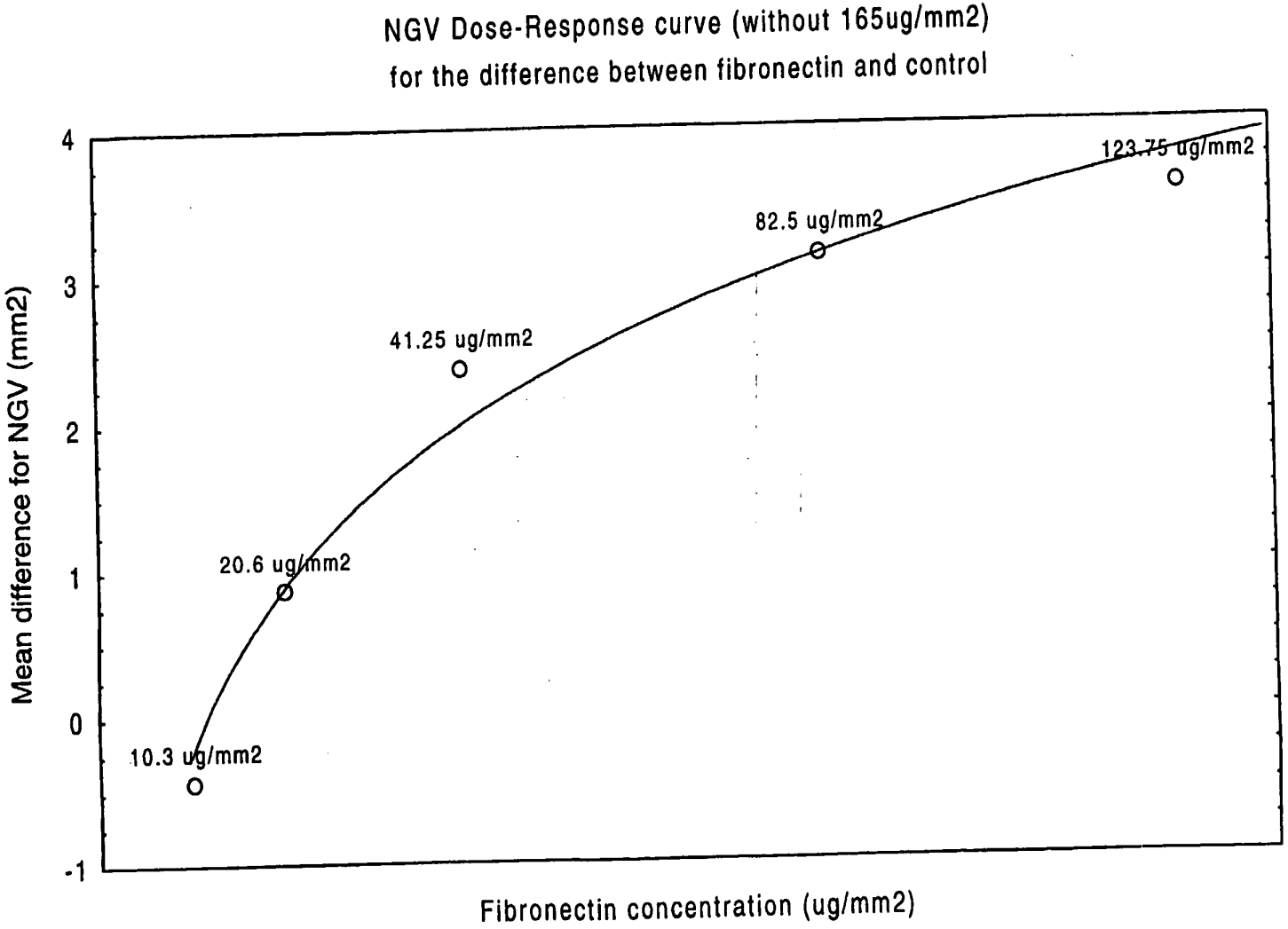

NGV Dose-Response Curve
for the difference between fibronectin and control.
Mean difference for NGV (mm2)
Fibronectin concentration (ug/mm2)
4
3
2
1
0
-1
123.25 ug/mm2
82.5 ug/mm2
41.25 ug/mm2
165 ug/mm2
20.6 ug/mm2
10.3 ug/mm2

Figure 16 shows that the 165 $\mu g/mm^2$ dose of fibronectin doesn't appear to be adequate for building a dose response curve. The low response for this high concentration shows that an external factor must be the cause.

Except for the highest concentration, the dose response curve shows a well behaved pattern.

Figure 17 shows the dose response curve without the highest fibronectin concentration.

Figure 17


NGV Dose-Response curve (without 165ug/mm2)
for the difference between fibronectin and control
Mean difference for NGV (mm2)
4
3
2
1
0
-1
10.3 ug/mm2
20.6 ug/mm2
41.25 ug/mm2
82.5 ug/mm2
123.75 ug/mm2
Fibronectin concentration (ug/mm2)

In the previous graphs the adjusted dose response curves were built with a simple difference between treatment and control.

Another way to present the adjusted dose response is with a relative difference between the treatment and the control. The relative difference (R.D.) is obtained by :

$$R.D. = \frac{(\text{Treatment mean} - \text{Control mean})*100}{(\text{Control mean})}$$

The percentage obtained can be plotted against fibronectin doses to produce dose "relative" response curves. Figures 18 and 19 present respectively such curves for MH M and NGV (without 165 ug/mm2).

Figure 18

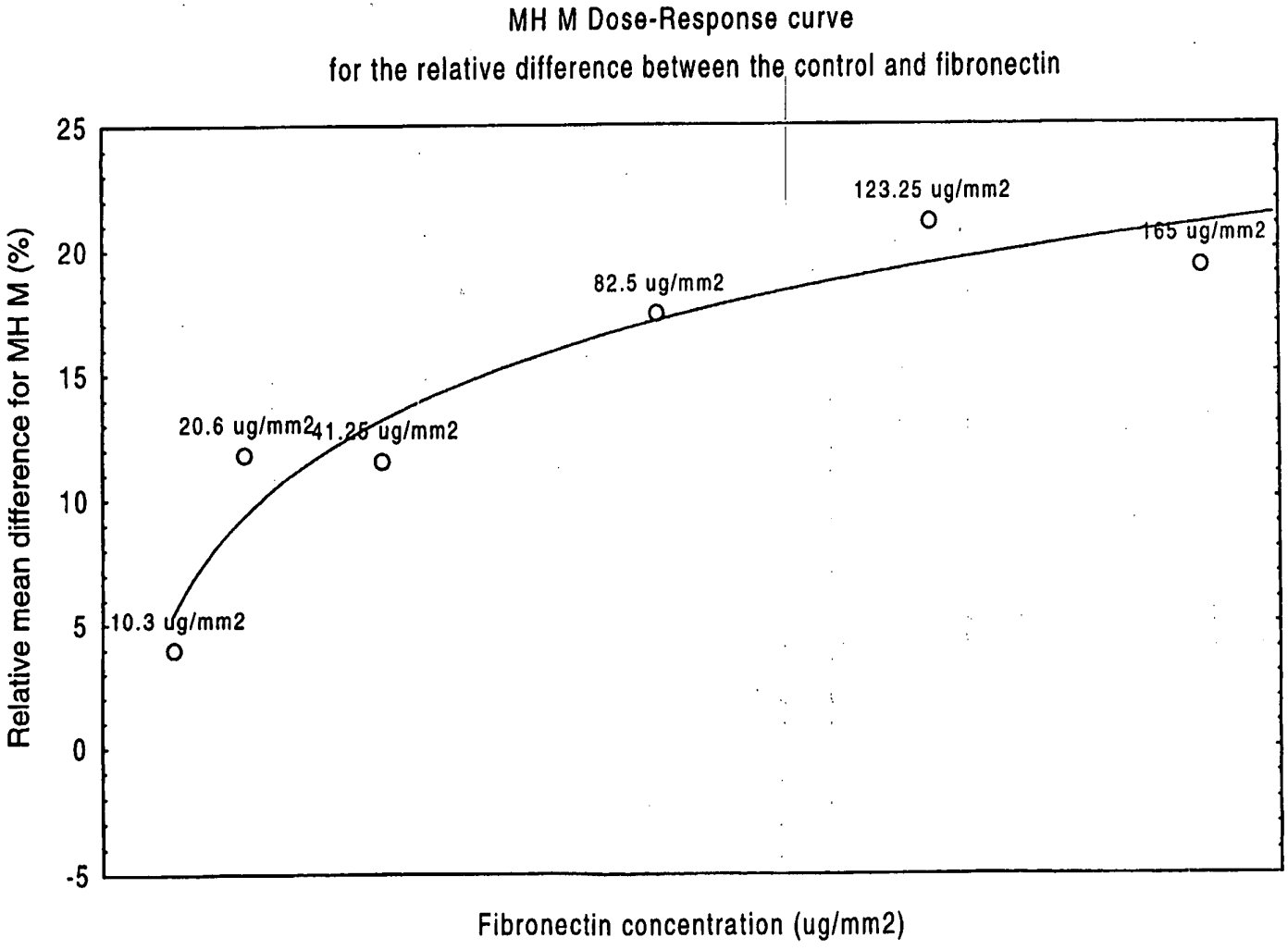
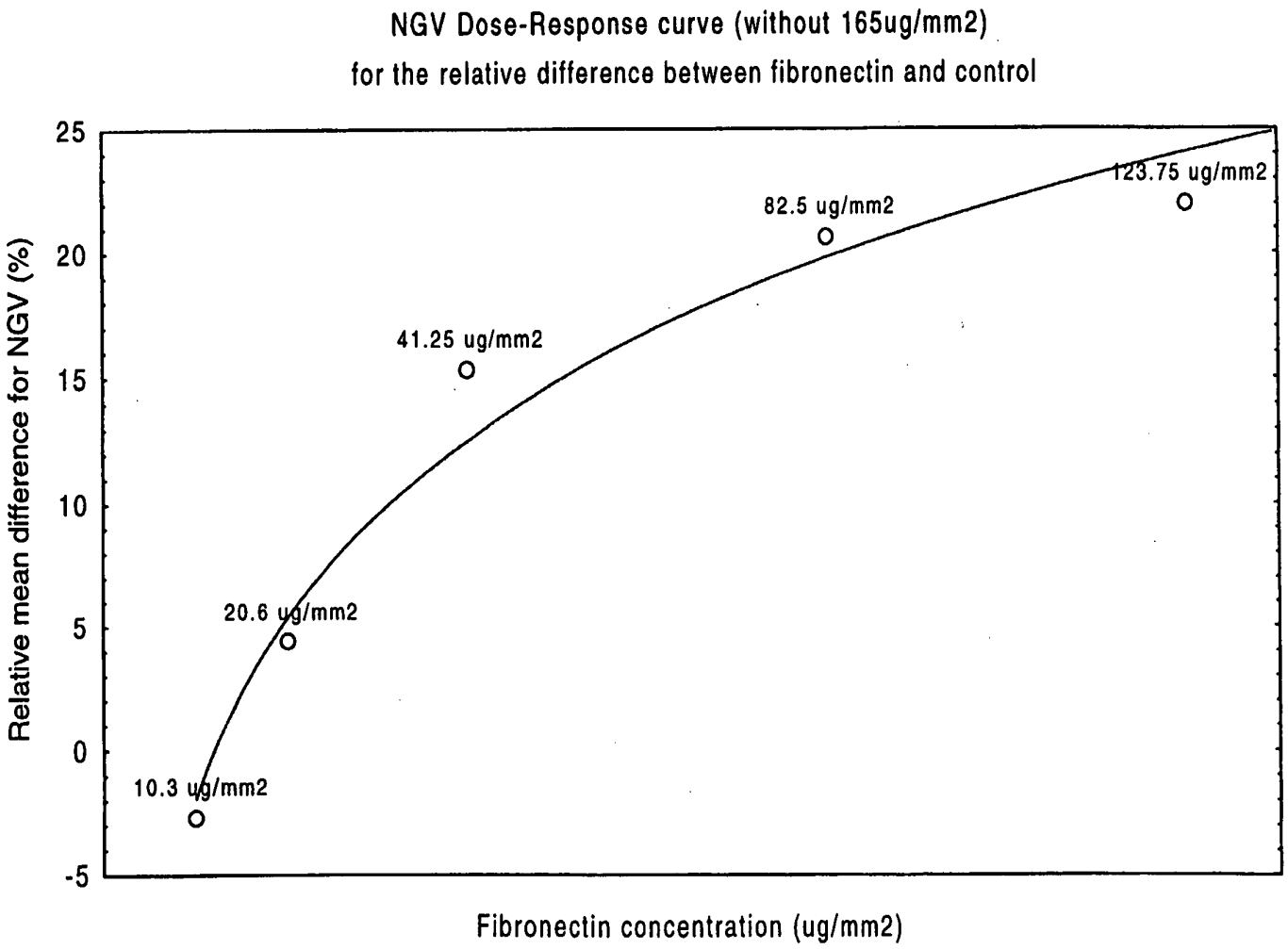


Figure 19


NGV Dose-Response curve (without 165ug/mm2)
for the relative difference between fibronectin and control
Relative mean difference for NGV (%)
25
20
15
10
5
0
-5
10.3 ug/mm2
20.6 ug/mm2
41.25 ug/mm2
82.5 ug/mm2
123.75 ug/mm2
Fibronectin concentration (ug/mm2)

Figures 18 and 19 show that the dose response curves for the relative differences are quite similar for MH M and NGV:

> The patterns are similar (given that 165 ug/mm2 is removed because the low response is not attributable to fibronectin)
>
> The plateau begins when the treatment is between 20% and 25% better than the control.

The conclusion is that Fibronectin has a significant effect on MH M and NGV. The effect increases with doses. The effect seems to reach a plateau. With high enough doses, the effect on MH M and NGV is more than 20% better than the control.

Study of the effect of DermaLink on wound healing using the Rabbit Ear Dermal Ulcer Model.

DERMACOR INC

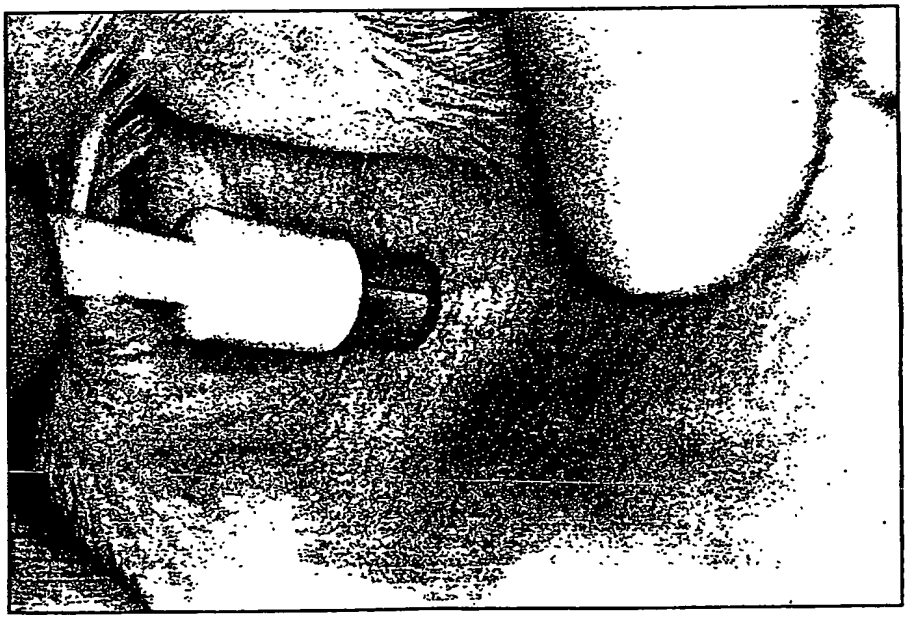
09

CONFIDENTIAL

In this model, dermal ulcers are created on the rabbit ear of New Zealand white rabbits with a 6 mm AcuPunch® biopsy punch

The dermis is removed and bare cartilage is exposed.

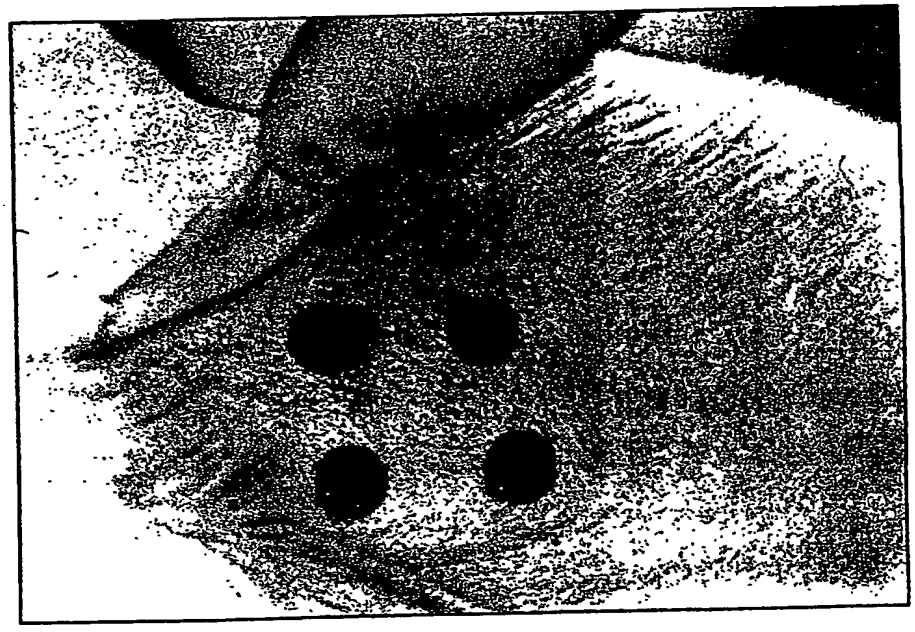
As cartilage is non-vascularized, new granulation tissue formation occurs only at the periphery of the ulcer.



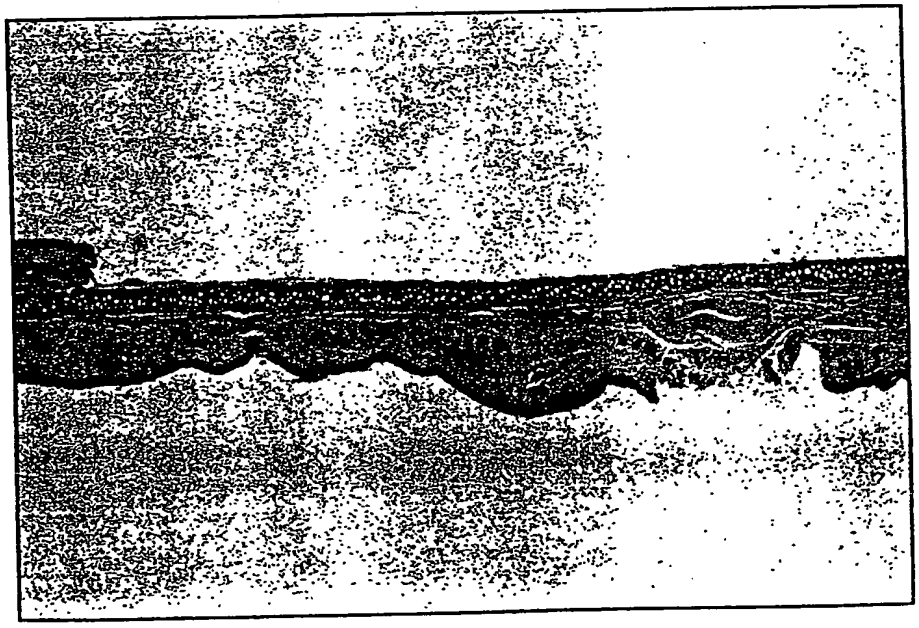
Four ulcers may be created per rabbit ear.

One ear is used for the active treatment sites and the other ear is used for the control sites.

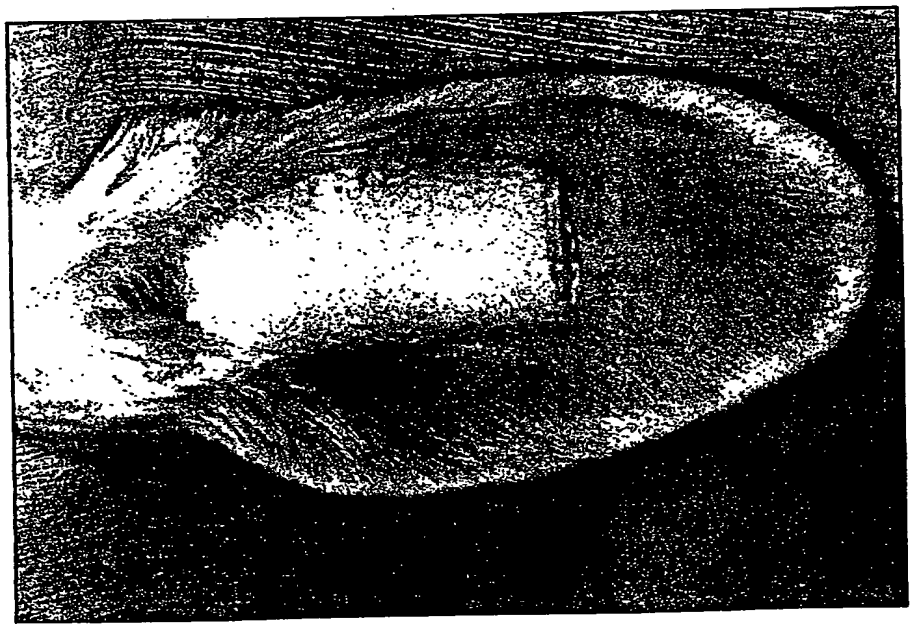
In this case, DermaLink (fibronectin-calcium-alginate dressing) was applied on the ulcers of one ear and calcium-alginate dressing without fibronectin was applied to ulcers on the other ear.



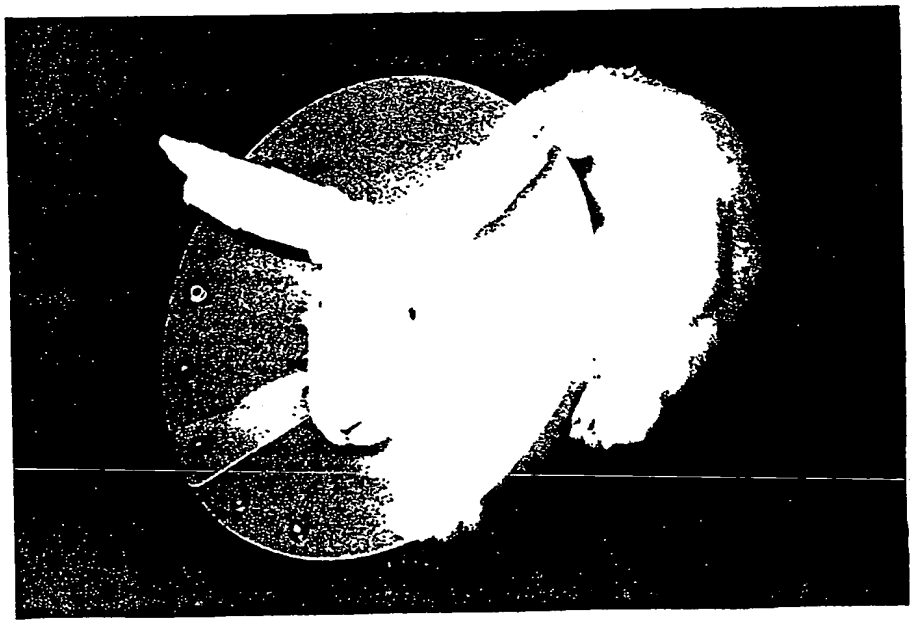
Histological section showing the bare cartilage exposed at day 0 of the treatment.



The wound dressing is applied, covered by a Tegaderm ™ dressing on top of which is applied a rolled gauze dressing. This dressing is left in place for a period of 7 to 10 days.



A neck collar is put in place to make sure the dressing is not disturbed.



Study

1- variables :

MH M (mm) : Mean Height Mean
NGV : New Granulation Volume
NGT GAP M : New Granulation Tissue GAP Mean

2- Between 12 and 13 rabbits studied by DermaLink dose.

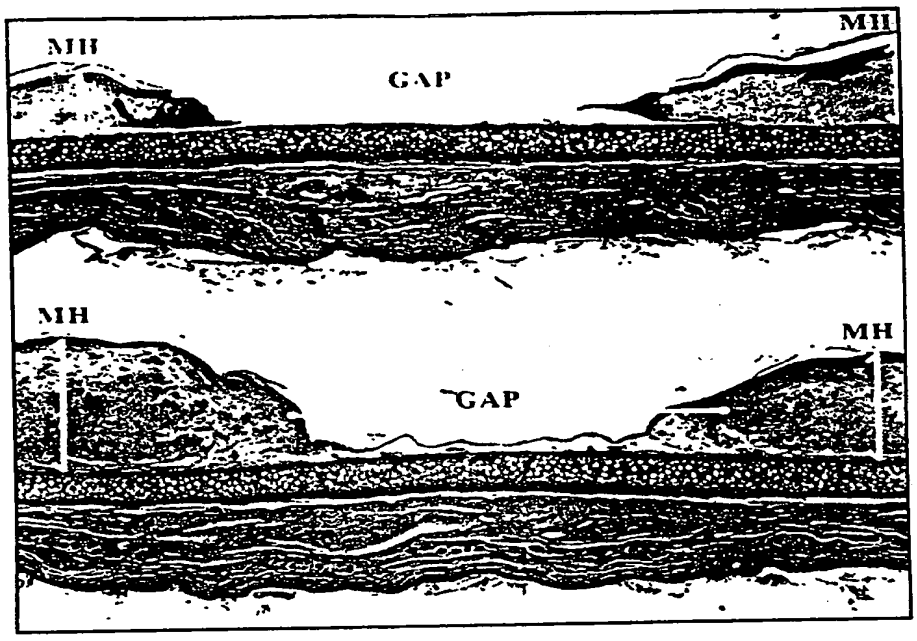
3- Six doses studied : 10.3, 20.6, 41.25, 82.5, 123.75 and 165 µg/mm²

- **Histomorphometric measurement of new granulation tissue**

- MH Mean (MH M) is mean height of granulation tissue.
- New Granulation Volume (NGV) is new granulation tissue area x MH
- MH M and NGV are measured after a period of treatment (7-10 days)

- GAP distance is used to measure tissue area of wound $(GAP/2)^2\pi$
- New granulation tissue area is area of wound at day 0 minus area of wound at day 7-10.

- **MH and GAP values are obtained by histomorphometric measurements using Biometrics Bioquant true color laser vision (R&M Nashville,TN)** light microscope with software to analyse hits histological tissue measurements



Summary of the Fibronectin effect on variable MH M

	Nb rabbits	Nb ulcers per group	Control group MH M mean (mm)	Treatment group MH M mean (mm)	p-value
10 3 µg/mm²	12	48	0 75	0 78	p=0 248
20 6 µg/mm²	13	52	0 76	0 85	p<0.0001
41 25 µg/mm²	12	48	0 69	0 77	p<0.0001
82 5 µg/mm²	13	52	0 67	0 79	p<0.0001
123 75 µg/mm²	12	48	0 69	0 82	p<0.0001
165 µg/mm²	12	48	0 66	0 80	p<0.0001

Summary of the Fibronectin effect on variable NGV

	Nb rabbits	Nb ulcers per group	Control group NGV mean (mm^3)	Treatment group NGV mean (mm^3)	p-value
10.3 µg/mm²	12	48	16 34	15.91	p=0 612
20.6 µg/mm²	13	52	19.66	20.53	p=0.282
41.25 µg/mm²	12	48	15.52	17 90	p=0.0008
82.5 µg/mm²	13	52	15.13	18 26	p<0.0001
123 75 µg/mm²	12	48	16.19	19 75	p<0.0001
165 µg/mm²	12	48	14 36	15 80	p=0 104

Dose Response

In order to show the dose response relation, two graphs are presented for each variable (MH M and NGV).

-A bar graph showing the response of both groups (control and fibronectin) for rabbits associated to each fibronectin dose.

-A scatterplot showing the dose response curve for the difference between the fibronectin and control responses.

Figure 1
Responses for control and fibronectin groups for variable MH M

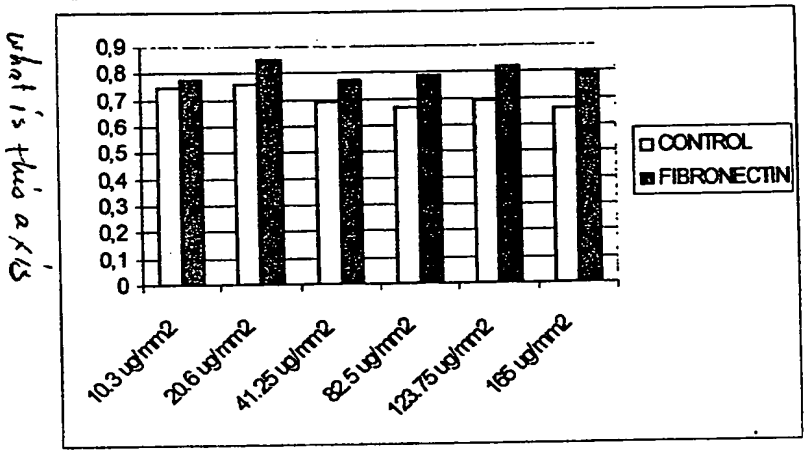


Figure 1 shows that the rabbits used for the 20.6 $\mu g/mm^2$ dose of fibronectin responded very highly to the fibronectin treatment.

However, the higher response for the control ulcers show that this group of rabbits probably had better healing possibilities.

This result explains why the dose response curve should be computed with the difference between the fibronectin and control groups of ulcers.

We can thus say that the dose response curves is adjusted for the rabbit effect.

Figure 2

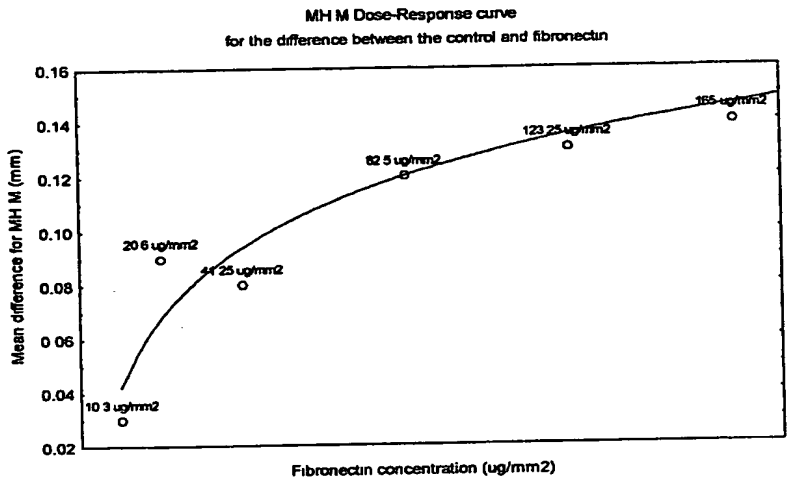


Figure 2 shows an adjusted dose response curve with the response increasing rapidly for small doses and reaching a plateau for the highest concentrations.

The use of an adjusted dose response curve is the most adequate way to represent the response of the rabbits to the treatment because :

-The control groups have different responses showing different intrinsic healing possibilities.
-The use of a paired experiment allow an adjustment for each subject.
-The difference between the control (placebo) and the treatment (fibronectin) is a good estimation of the real effect of the treatment.

Without the use of an adjusted dose response curve, we would conclude that the optimal use of fibronectin is at a 20.6 ug/mm2 dose since higher concentrations show a lower absolute response on MH M. However, the difference between the control and treatment group is not significant at that dose. The adjusted dose response curve indicates that even if the absolute response for 165 ug/mm2 is smaller that the absolute response for 20.6 ug/mm2, the intrinsic characteristics showed by the control groups results show that the absolute response for 165 ug/mm2 would have been higher if similar group of rabbits were used.

Figure 3
Responses for control and fibronectin groups for variable NGV

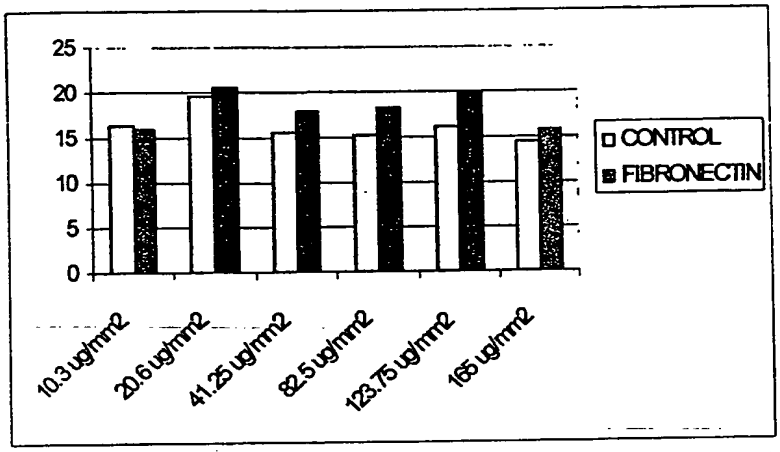


Figure 3 shows that the rabbits used for the 20.6 $\mu g/mm^2$ dose of fibronectin responded very highly to the fibronectin treatment.

However, the higher response for the control ulcers show that this group of rabbits probably had better healing possibilities.

This result explains why the dose response curve should be computed with the difference between the fibronectin and control groups of ulcers.

We can thus say that the dose response curves is adjusted for the rabbit effect.

Figure 4

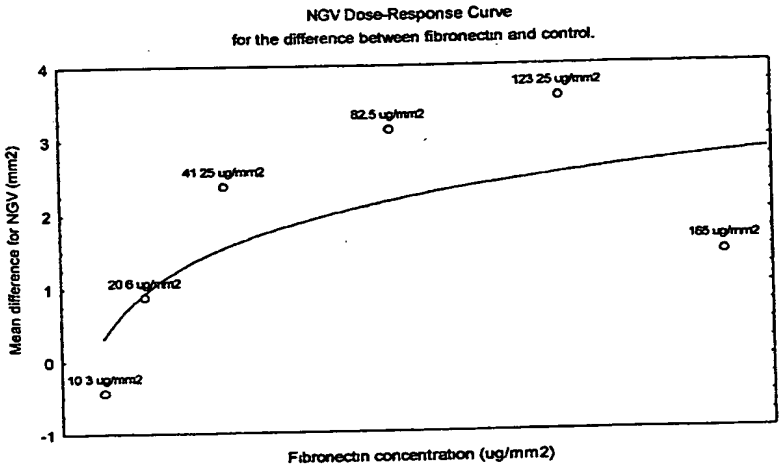
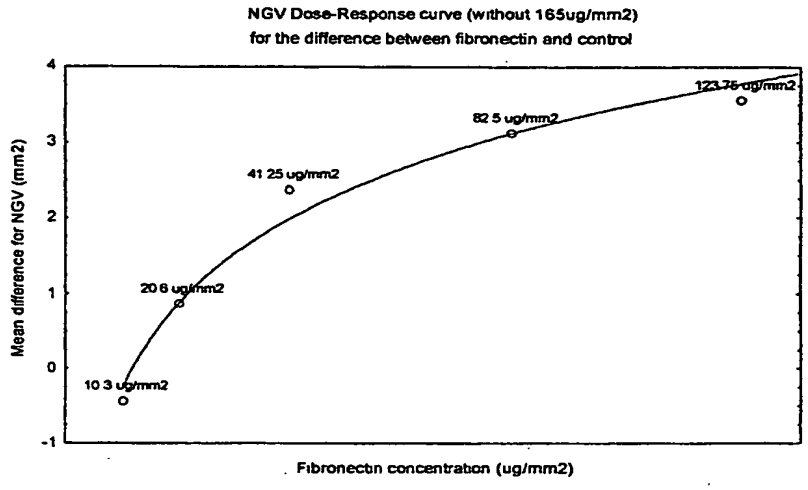


Figure 4 shows that the 165 μg/mm² dose of fibronectin doesn't appear to be adequate for building a dose response curve. The low response for this high concentration shows that an external factor must be the cause.

Except for the highest concentration, the dose response curve shows a well behaved pattern.

Figure 5 shows the dose response curve without the highest fibronectin concentration.

Figure 5



In the previous graphs the adjusted dose response curves were built with a simple difference between treatment and control.

Another way to present the adjusted dose response is with a relative difference between the treatment and the control. The relative difference (R.D.) is obtained by :

$$R.D. = \frac{(\text{Treatment mean} - \text{Control mean}) * 100}{(\text{Control mean})}$$

The percentage obtained can be plotted against fibronectin doses to produce dose "relative" response curves. Figures 6 and 7 present respectively such curves for MH M and NGV (without 165 ug/mm2).

Figure 6

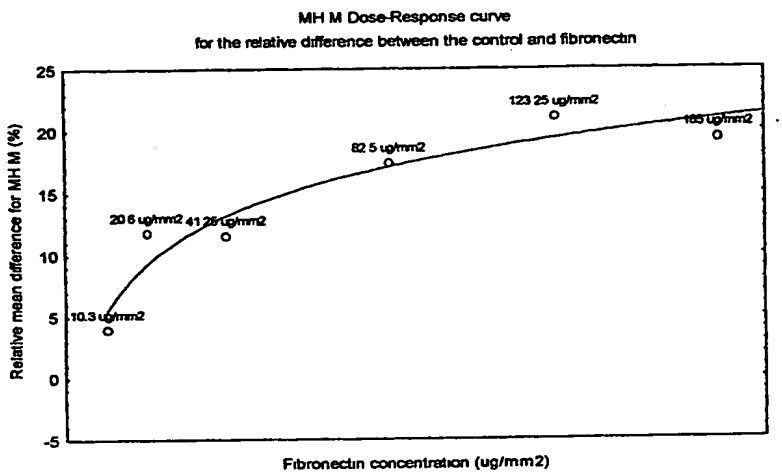
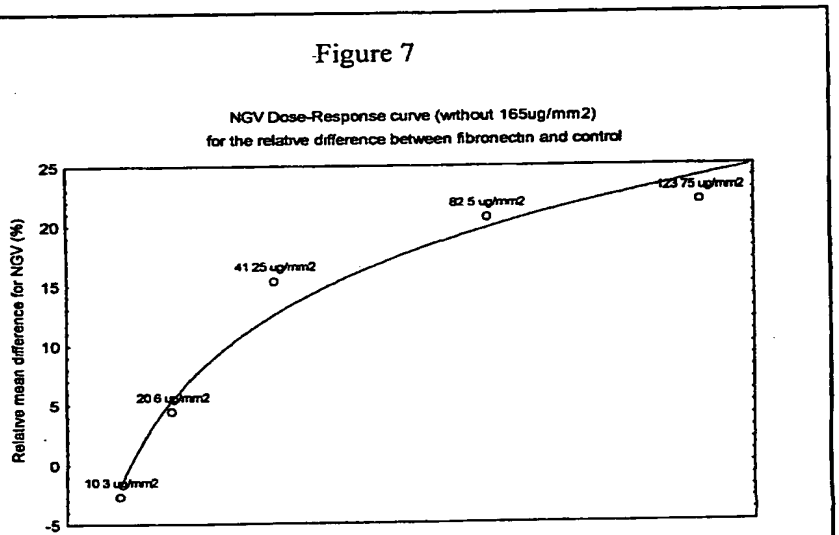


Figure 7



Figures 6 and 7 show that the dose response curves for the relative differences are quite similar for MH M and NGV:

The patterns are similar (given that 165 ug/mm2 is removed because the low response is not attributable to fibronectin)

The plateau begins when the treatment is between 20% and 25% better than the control.

The conclusion is that Fibronectin has a significant effect on MH M and NGV. The effect increases with doses. The effect seems to reach a plateau. With high enough doses, the effect on MH M and NGV is more than 20% better than the control.

.17 to 11

Composition of fibronectin carbomer hydrogels

Stock solution of carbomer			
3.75 %		2.80 %	
10 g (0.5% FN/ 0.28 % Carbomer)	10 g (1.0% FN/0.28 % Carbomer)	**10 g (0.5% FN/ 0.28 % Carbomer)**	**10 g (1.0% FN/0.28 % Carbomer)**
0.75 g Carbomer	0.75 g Carbomer	**1.00 g Carbomer**	**1.00 g Carbomer**
0.05 g FN	0.1 g FN	**0.05 g FN**	**0.1 g FN**
9.106 g water	9.056 g water	**8.856 g water**	**8.806 g water**
0.094 g NaOH 3M	0.094 g NaOH 3M	**0.094 g NaOH 3M**	**0.094 g NaOH 3M**

Stock solution of carbomer			
3.75 %		2.80 %	
20 g (0.5% FN/ 0.28 % Carbomer)	20 g (1.0% FN/0.28 % Carbomer)	**20 g (0.5% FN/ 0.28 % Carbomer)**	**20 g (1.0% FN/0.28 % Carbomer)**
1.50 g Carbomer	1.50 g Carbomer	**2.00 g Carbomer**	**2.00 g Carbomer**
0.1 g FN	0.2 g FN	**0.1 g FN**	**0.2 g FN**
18.212 g water	18.112 g water	**17.712 g water**	**17.612 g water**
0.188 g NaOH 3M	0.188 g NaOH 3 M	**0.188 g NaOH 3M**	**0.188 g NaOH 3M**

In order to prepare the fibronectin gel, the following ingredients must be added in this sequence. First, the pH of demineralized water (8.856 or 8.806 grams) is adjusted at 11.6 with 0.0235 grams of NaOH 3M. Lyophilized fibronectin is next dissolved in demineralized basic water (0.05 or 0.1 grams). In a final step of the procedure, 0.028 grams of carbomer and 0.0705 grams of NaOH 3M are added to the mixture. Please note the 0.028 grams of carbomer comes from 1.0 gram of carbomer stock solution of 2.8%. Please also note that the concentrated Carbomer start stock solution may be at 3.75% or 2.80%. This is illustrated in the right part of the table.

Tap water is demineralized using a demineralizer with Millipore Milli-Q Water System

For preparing 10 g of fibronectin carbomer hydrogel, the following ingredients must be added in sequence. First, the pH of 8.8 mL demineralized water pH 5.0 is adjusted at pH 8.0 to 11.0 with the addition of 2.95 µg to 2.95 mg NaOH 3M. The lyophilized fibronectin is next dissolved in demineralized water pH 8.0 to 11.0 in quantities varying from 0.05 to 0.1 g. In a final step of the procedure, 1 mL of water containing 0.028 g of carbopol and varying amount of NaOH 3M from 0.09399705 g to 0.09105 g are added to the mixture. (from Annie)

Pr duct : DermaLink
Protoc l : FN 99-01

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SPONSOR :

Dermacor Inc.
2795, boul. Laurier, bur. 200
Sainte-Foy (Québec)
G1V 4M7

TITLE :

A double-blind, vehicle-controlled, randomized study to assess, in combination with high compression therapy, the efficacy and safety of homologous human plasma fibronectin applied topically in a calcium-alginate dressing (DermaLink), compared to a calcium-alginate dressing without fibronectin (the vehicle), in the treatment of chronic venous ulcers of the lower limb.

INVESTIGATOR (S) :

PRIMARY :

SECONDARY : / SUBINVESTIGATOR :

SITE :

INSTITUTIONAL REVIEW BOARD/ETHICAL REVIEW COMMITTEE :

77

PROTOCOL SYNOPSIS

Protocol Number	FN 99-01
Title	A double-blind, vehicle-controlled, randomized study to assess, in combination with high compression therapy, the efficacy and safety of homologous human plasma fibronectin applied topically in a calcium-alginate dressing (DermaLink), compared to a calcium-alginate dressing without fibronectin (the vehicle), in the treatment of chronic venous ulcers of the lower limb.
STUDY DESIGN	Multi-centre, randomized, double-blind, vehicle-controlled
TREATMENT	Twice weekly application of fibronectin embodied in a calcium-alginate dressing (Dermalink) or calcium-alginate dressing alone (vehicle) to a chronic venous ulcer
PRIMARY ENDPOINT	To determine the mean (median) time required for complete healing with DermaLink vs vehicle-control
SECONDARY ENDPOINTS	1. To determine the proportion of patients who experience complete (100 %) closure of their venous ulcer with DermaLink vs vehicle–control. 2. To determine the mean (median) area of ulcer in patients treated with DermaLink vs vehicle-control 3. To assess granulation tissue formation in patients treated with DermaLink vs vehicle-control 4. To assess the safety of DermaLink vs vehicle-control
SITES	10-12 Clinical Centres in Canada and The United States
NUMBER OF PATIENTS	126 enrolled, 105 completing
PATIENT POPULATION	Male or female patients diagnosed with a chronic venous leg ulcer present for at least three months. Patients will be randomized into one of two treatment arms : ❑ Control group : twice a week treatment with vehicle only; ❑ Treatment group : twice a week treatment with DermaLink at a concentration of 80 µg/mm2 of ulcer surface area.
EXPECTED ENROLLMENT RATE	1-2 patients / site / month
ESTIMATED CRF PAGES	120
PATIENT PARTICIPATION	Each evaluable subject will be asked to respect the following visit schedule for a total of 36 visits : ❑ Eight visits during observation phase I, four weeks prior to randomization; ❑ Twice weekly visits during a twelve week active treatment phase (or until complete re-epithelization, withdrawal or termination); ❑ Three visits during observation phase II, once a month for three months.
MONITORING FREQUENCY	Approximately once every four to six weeks, or as required by site enrollment

ABSTRACT

This is a randomized, double blind, vehicl -controlled trial of the safety and efficacy of a topical dosage form containing fibronectin in the treatment of chronic venous leg ulcers. Fibronectin embodied in a calcium-alginate dressing (DermaLink) or calcium-alginate dressing alone (the vehicle) will be applied twice weekly, in combination with high compression therapy, on a chronic venous ulcer that has been present for at least three months. Patients will be assessed twice per week until complete reepithelialization, withdrawal from the study, or study termination.

THE HYPOTHESIS

By administrating fibronectin into the chronic wound by way of a pharmaceutical preparation that ensures effective delivery, the normal processes of wound healing will be initiated (or re-initiated) and possibly accelerated.

BACKGROUND AND RATIONALE

1- The physiological role of fibronectin in wound healing

Fibronectin is a plasma and ubiquitous extracellular glycoprotein containing around 5% carbohydrate. It exists in a soluble form in body.fluids and in an insoluble form in the extracellular matrix. Normally found in plasma at a concentration of about 300 µg/mL, fibronectin can be extracted for therapeutic purposes from human plasma under viral-safe conditions (1).

Fibronectin was shown to play a major role in many important physiological processes, such as embryogenesis, hemostasis, thrombosis and wound healing (for review see 2). Plasma fibronectin is also known by various other names, including cold-insoluble globulin, antigelatin factor, cell attachment protein, cell spreading factor, and opsonic a2-surface binding glycoprotein. These names reflect biological activities of fibronectin such as cell recruitment and attachment, opsonization of particulate debris, and collagen binding activity (for review see 3).

Wound healing is usually divided into three phases: the inflammatory phase, the proliferative phase, and the remodeling phase. These phases overlap during the whole duration of wound healing. Fibronectin is believed to play a variety of critical roles in all of these phases. Reviews on wound healing and the potential roles of fibronectin have been published. (4, 5)

The biological properties by which fibronectin may exert it's biological effects on wound healing are the following:

1- Chemotactic factor activity for wound healing cells
2- Opsonin activity (debridement activity) for necrotic cell debris
3- Ability to act as a substratum for cell movement and spreading of wound healing cells
4- Role in final matrix deposition and assembly.

The chemotactic activity of fibronectin for monocytes (6,7) is potentially important in the early acute inflammatory phase of wound healing. It is known that these cells produce growth factors at the wound site which in turn will have chemotactic and growth stimulating activities for fibroblasts, endothelial cells and epithelial cells. Moreover, fibronectin was shown to exert, by itself, chemotactic activity for

fibroblasts (8-10) and endothelial cells (11). The importance of fibronectin for keratinocyte attachment and spreading was also clearly established (12-14). Since the migration and proliferation of fibroblasts, endothelial and epithelial cells at the wound site is crucial for tissue repair, fibronectin is therefore likely to play an important role in the transition of the wound repair process from the acute inflammatory phase to the proliferative phase. The phagocytosis of bacteria and necrotic tissue debris are also important events for wound healing in the acute inflammatory phase. Although it has not been clearly established whether or not the opsonin activity of fibronectin contributes to the phagocytosis of bacteria, fibronectin is believed to be involved in stimulating the phagocytosis of necrotic tissue debris (15-18).

Fibronectin also acts as a cell substratum for fibroblasts (19), endothelial (20-22) and epithelial cells (23-25), as well as for macrophages (26,27) during wound repair. This biological property of fibronectin not only assists these cells in their movement from surrounding normal tissues to the wound, but also helps them to anchor in tissues once at the wound site. Fibronectin was found to be abundant around fibroblasts, epidermal and endothelial cells during the wound healing process but to disappear once this process is completed. It is therefore believed that fibronectin provides a provisional matrix for cells actively involved in new collagen production, angiogenesis and epidermization (28-31). Finally, from immunohistochemical staining studies on granulating tissues and cultured fibroblasts, evidence was obtained that fibronectin acts as a scaffold for collagen deposition since there is temporary co-deposition of fibronectin with components of the extracellular matrix such as collagen and proteoglycans (32-35). This would suggest that fibronectin is also important in the final tissue assembly and remodeling phase of wound healing. Finally, although fibronectin was not clearly shown to have the ability to act as a growth factor, it was demonstrated to play a central role in TGF β-induced cell fibroplasia (36).

In summary, all of the evidence and observations described above, argue in favor of a central role for fibronectin in wound healing.

2- The efficacy of fibronectin in animal models of wound healing

A number of studies have demonstrated the efficacy of fibronectin in stimulating wound healing in various animal models. These include two rat skin models (37,38), and two rabbit corneal ulcer models (39,40). More recently, Dermacor has conducted experiments using the rabbit ear acute dermal wound model developed by Mustoe et al. (41). A detailed description of the model and the findings are presented in the Investigational Brochure accompanying this protocol. In this model, dermal ulcers are created on the rabbit ear of New Zealand white rabbits with a 6 mm punch biopsy. The dermis is removed and bare cartilage is exposed. Four wounds may be created per ear. As cartilage is non-vascularized, new granulation tissue formation occurs only at the periphery of the ulcer. One ear is used for the active treatment sites and the other ear is used for the control site. In this case, DermaLink was applied on the wounds of one ear and calcium-alginate dressing without fibronectin was applied to ulcers on the other ear. The wound dressing, once applied, was covered by a Tegaderm™ dressing on top of which was applied a rolled gauze dressing. This dressing was left in place for a period of 8 days. A neck collar was then put in place to make sure the dressing was not disturbed. The studied variables were the Mean Height Mean of new granulation tissue (MH M in mm) at the wound periphery and New Granulation Volume (NGV in mm^3). Results showed a dose response effect of fibronectin compared to the vehicle alone over doses ranging from 10 to 120 ug/ of fibronectin per mm^2 of wound surface area. With the 80 and 120 ug/mm^2 doses, both MH M and NGV were 20% higher than the control with P values of <0.0001 in both cases.

3-Venous stasis leg ulcers.

Chronic ulcers of the lower limb have a prevalence between 0.18% to 1.3% in the general population (42-45). Approximately 40% to 70% of these are secondary to chronic venous insufficiency

(46,47). The most successful form of treatment is high compression th rapy (for recent reviews see 48,49). It is estimated that when appropriately administered, between 30% to 60% of patients will heal completely within a period of 6 months (50). Although others have reported higher rates for patients treated in a specific treatment center (51), their appears t be a general consensus for the 30 % to 60 % rates among various treatment centers. The need to reduce the time to heal, as well as to increase th proportion of patients that heal over any given period of time are primary objectives of care givers. It should be stressed that the recurrence rate for these chronic wounds can be as high as 70% (50) and that the average treatment cost for a given patient was estimated to be $9685, with a median of $3036 (52). The need to find treatment modalities that add to the efficacy of high compression therapy also comes from factors such as reduced quality of life of the patient and the total burden placed on society due to loss in productivity.

PREVIOUS EXPERIENCE WITH FIBRONECTIN IN TREATING CHRONIC ULCERS IN HUMANS.

In small clinical trials, topically applied plasma fibronectin has been reported as being potentially useful for increasing the rate of wound healing such as in corneal wounds (53,54) and leg ulcers (55). However, there is no suitable topical carrier for use in treating wounds that can ensure delivery of an effective amount of fibronectin in a pharmaceutically acceptable formulation. A major limiting factor in developing an effective topical dosage form of a drug is not only having an active drug, but also having a formulation that allows the passage of the active drug from the carrier into a site of delivery with optimal wound contact time. Dermacor has worked in developing such a formulation and the various studies conducted in humans during the development of such a formulation are described elsewhere (56 and Investigational Brochure). All studies, except one, are anecdotal and are non- controlled.

A pilot, randomized and placebo-controlled clinical trial was conducted in a small group of patients with chronic venous leg ulcers, comparing a carbomer hydrogel containing no fibronectin to a carbomer hydrogel embodying 0.2% fibronectin. This preparation delivered in the wound 0.4 ug of fibronectin per mm^2 of wound surface area. This preparation and concentration in fibronectin was used because at the time of the study, the technology to produce formulations with higher concentrations had not been developed. All patients entered had a venous leg ulcer of at least three months duration and had failed to respond to at least two different treatment modalities. A detailed description of this study can be found in the Investigational Brochure accompanying this protocol.

Outcome of the clinical trial: No adverse event was found to be related to the 0.2% fibronectin carbomer hydrogel. Efficacy data analysis, from a statistical standpoint of view, was not reliable given the small sample size. Twenty patients were in the 0.2% fibronectin carbomer hydrogel group and 9 patients formed the carbomer hydrogel alone group. A comparable proportion of patients had at least an 80% decrease in wound size in both treatment groups: 10/20 for the active treatment group and 5/9 in the control group. However, in these responding patients, chronicity of wound at study entry in the fibronectin group was much higher than in the control group. Mean duration of wound prior to randomization was 13.4 ± 10.3 months for the fibronectin group and only 4.6 ± 1.2 for the control group. Since chronicity of wound is likely to be an important factor negatively affecting a treatment response, the positive response of patients with very chronic wounds in the fibronectin group supports the hypothesis that fibronectin can be beneficial for wound healing. It is also possible that wounds of low chronicity, such as that observed in the control group, are more prone to heal spontaneously, making treatment outcome in both groups difficult to compare. Furthermore, by providing a moist and absorbent environment to the wound, hydrogels alone could have some beneficial effect, making the population of patients treated with carbomer hydrogel alone not a true placebo control group. Finally, it must be stressed that the amount of fibronectin delivered in the wound (0.4 ug of fibronectin per mm^2 of wound surface area) could have been suboptimal and that a greater proportion of patients might have responded to a higher dose.

The present protocol will study a new formulation, DermaLink, which is a preparation of calcium-alginate in which is embodied fibronectin. This formulation has the ability to deliver 80 ug of fibronectin per mm^2 of wound surface area.

STUDY OBJECTIVES

Primary Objective

To evaluate the efficacy of DermaLink by comparing the mean (median) time required for complete healing of the chronic venous leg ulcer in patients that are treated with DermaLink as compared to the vehicle group.

Secondary Objectives

1. To determine the proportion of patients who experience complete (100 %) closure of their venous ulcer with DermaLink vs vehicle–control.
2. To determine the mean (median) area of ulcer healing in patients treated with DermaLink versus that of the vehicle-controlled preparation.
3. To assess granulation tissue formation in patients treated with DermaLink vs vehicle-control
4. To assess the safety of DermaLink vs vehicle-control

STUDY DESIGN

Overall design and control methods

This is a multi-center, randomized, double blind, vehicle-controlled study. Each subject will be assessed whenever possible by the same dermatologist and/or the same nurse at the following visits twice per week during four weeks before the randomization (observation phase I), twice per week during a twelve weeks active treatment phase, and once a month during 3 months after treatment completion (observation phase II).

The patients will be randomized into the two following groups:

1) Control group: patients randomized into this group will receive the vehicle in combination with high compression therapy as described in the treatment section.
2) Treatment group: patients randomized into this group will receive DermaLink at a concentration of 80 $\mu g/mm^2$ of ulcer surface area in combination with high compression therapy twice a week as described in the treatment section.

STUDY CENTERS

This is a multi-center trial that will be conducted in approximately 10-12 clinical centers in Canada and in the United States. These study sites are anticipated to be large referral centers experienced in the treatment of venous ulcers.

STUDY DURATION

The minimal duration of this study is 28 weeks, including a four-week observation phase I, a 12-week treatment phase followed by a 3 months observation phase II. During the treatment phase, patients will be followed twice per week in the clinic until either the endpoint is reached (complete wound closure) or the treatment phase is completed. Twice per week visits are scheduled for up to four months, with three additional observational visits (once a month for three months). Although DermaLink and the control vehicle *will* be applied to the wound twice per week, ulcer surface measurements will be performed every two weeks.

NUMBER OF SUBJECTS

Fifty subjects in each of three groups (150 total) will be recruited into this clinical study. The enrollment will include men and women at least 18 years old, presenting with venous insufficiency and chronic venous leg ulcers. The justification for the sample size is included in the statistical methods section of this document.

SUBJECT SELECTION

Inclusion criteria:

1. Subject presenting with a chronic venous ulcer of the lower limb, i.e., an ulcer appearing between the ankle and knee of patients with clinically confirmed venous disease. The following definition of chronic will be used: ulcer must be present for at least three (3) to thirty six (36) months prior to entry on this study.

2. The ulcer must be between 2-10 cm^2 of integrated surface.

3. Subject must have had adequate compression therapy of the ulcer (Doppler ABI 0.7 or more.) for a period of at least four weeks prior to randomization

4. Subject is able to tolerate high compression therapy.

5. Subject must be ambulatory.

6. Subject must be > 18 years of age.

7. Except venous stasis ulcers, the patient is judged to not have a coexisting illness or medication that will significantly impair ulcer healing.

8. Female patient of childbearing potential must demonstrate a serum B-HCG level consistent with a non-gravid state at visit1 and a negative urine B-HCG test at visit 9 (randomization) and agree to remain abstinent, use double-barrier contraception (partner using condom and

patient using diaphragm contraceptive sponge, or IUD) beginning at least 7 days prior to treatment and continuing at least 14 days after end of treatment. Women who are postmenopausal or status posthysterectomy or tubal ligation are exempt from this requirement (postmenopausal is defined as no menses for the previous one year. If cessation of menses is within 18 months, FSH must be documented as elevated into the postmenopausal range prestudy.)

9. Subject must be able to give written informed consent.

10. Subject must be willing to participate in the study and follow up.

Exclusion Criteria

1. Subjects who are currently participating in another clinical trial with any investigational drug or device.

2. Subject whose ulcer surface area has decreased ≥ than 30 % during the four week period prior to randomisation

3. Excessive exudation of the ulcer as defined by a need of more than one change of dressing per week during the last two weeks before randomization (visits 5 to 8).

4. Subjects with a known allergy to calcium-alginate

5. Subjects presenting an ulcer with an exposed bone and/or tendon and/or muscle.

6. Bacterial bioburden 4 weeks prior to randomisation reveals the presence of streptoccocus and/or a bacterial count ≥ 1×10^6 organisms per gram of tissue.

7. Ulcer treatment with bioengeneered skin products or growth factors during the 6 weeks prior randomization.

8. Any evidence of active infection of the ulcer including fever, pain, abnormal erythema or increased warmth or edema of the wound bed or surrounding tissue, lymphadenitis or excessive discharge.

9. Lactating women

10. Subjects with medical, surgical, and/or dermatological disorders that might interfere with the course of the ulcer such as:

    - Vasculitis
    - Lower limb arterial insufficiency (ankle/arm index less than 70% or local Doppler pressure less than 70 mm Hg (in absolute value)
    - Clinical hypothyroidism
    - Abnormal nutritional status as defined by a serum albumin test < 20 g/L and/or iron deficiency anemia (hemoglobine < 90 g / L)
    - Uncontrolled diabetes as defined by above normal level of glycosylated hemoglobin (HbA_{1c} > 10 %)
    - Administration of Prednisone (more than 7.5mg/day)

- Other topical medication for the ulcer
- Currently alcohol or other substance abuse

11. The patient has evidence of impaired renal function defined as a serum creatinine greater than 2 times above normal value.

12. The patient has angina or congestive heart failure with symptoms that occur at rest or minimal activity. (*Note : patients with a history of myocardial infarction or coronary arterial bypass grafting more than 1 year prior to study start may participate. However, if a patient develops unstable angina or a myocardial infarction during the study.they must be discontinued from the study.)*

13. The patient has uncontrolled hypertension (diastolic blood pressure ≥ 120 mm Hg).

14. The patient has active hepatitis/hepatic disease as defined by liver function tests (SGOT, SGPT) above 3 X upper limit of normal.

15. Patient with uncontrolled neoplasia which in the opinion of the investigator will interfer with the outcome of the trial.

16. Patient has morbid obesity as defined by a non-ambulatory status.

17. The patient is, in the opinion of the investigator legally or mentally incapacitated such that informed consent cannot be obtained or the patient cannot read or comprehend written material.

STUDY TREATMENT

Source Material for DermaLink and vehicle

DermaLink consists of human plasma fibronectin lyophilized in the presence of calcium-alginate so as to form solid disks which are applied topically to the wound surface. As compared with delivery of liquid fibronectin, the calcium-alginate disks help assure the delivery of known, consistent amounts of fibronectin to the wound surface since the alginate contains a known quantity of fibronectin per cm^2 and it dissolves readily following wetting with sterile saline.

Fibronectin is obtained from cryoprecipitate purchased from licensed manufacturers of human blood plasma products. Following dissolution and clarification, cryoprecipitate extract is treated to inactivate lipid enveloped viruses by the solvent/detergent procedure using 0.3% tri (n-butyl) phosphate (TNBP) and 1% Triton X-100. The process reagents are removed and the fibronectin is purified by gelatin-Sepharose affinity chromatography, eluting bound fibronectin from the column with 1 M potassium bromide at pH 5.0. Following removal of salts, the fibronectin is sterilized by membrane filtration, combined with sterile calcium-alginate, and filled aseptically under class 100 conditions into sterile glass vials. Stoppers are inserted partway into the vials, and the preparation is dried by lyophilization. On use, the fibronectin/calcium-alginate disks are removed from the vial by forceps, placed on the wound to be treated, and wetted by sterile saline obtained from the hospital pharmacy.

Source Material for DermaLink Delivery System

DermaLink delivery system consists of human plasma fibronectin lyophilized in the presence of calcium-alginate to form solid disks, which are then applied topically to the wound surface to be treated. As opposed to liquid formulations containing fibronectin, the use of calcium-alginate disks helps insure the delivery of known, consistent amounts of fibronectin to a wound surface, since calcium-alginate disks contain a known quantity of fibronectin per cm^2 and will dissolve readily after wetting with sterile saline.

Fibronectin is obtained from cryoprecipitate purchased from licensed manufacturers (MedImmune, Maryland through DCI Management, New York, NY). The cryoprecipitate extract is first dissolved and clarified. The cryoprecipitate extract is then treated to inactivate lipid enveloped viruses by the solvent/detergent procedure using 0.3% tri (n-butyl) phosphate (TNBP) and 1% Triton X-100 for four hours at room temperature. The process reagents are removed and the fibronectin is purified on a gelatin-Sepharose affinity chromatography. Bound fibronectin is eluted from the column with 1M potassium bromide at pH 5.0. Following the removal of salts, the fibronectin is sterilized by 100 KD membrane ultrafiltration and combined with sterile calcium-alginate. Sterile glass vials are filled aseptically with the fibronectin/calcium alginate mixture under Food and Drug Administration, class 100 good manufacturing procedures. Stoppers are inserted partway into the vials and the preparation is dried on a FTS Duralyophilizer lyophilizer. To use, the fibronectin/calcium-alginate disks are removed from the vial by forceps, placed on the wound to be treated and wetted with sterile saline obtained from the hospital pharmacy.

Descripti n f Topical dosage f rm, Dose, Route, and Duration of Study Treatment

The topical dosage form will consist of a fibronectin calcium-alginate sponge-like dressing with a disk shape (DermaLink). Size will be 5 cm^2 in diameter. More than one dressing may be used depending on ulcer size and must be trimmed to fit the shape of the wound. The dressing will deliver 80 µg per mm^2 of ulcer surface area. Once the dressing is applied, it will be wetted with 0.9% saline. The vehicle dressing will be constituted in exactly the same way except for the presence of fibronectin. The study and control dressings will be marked by a code (i.e., a series of numbers). When the patient is randomized, the study coordinator will be given that code which will correspond to the randomized treatment assignment. The study coordinator, principal investigator, and patient remain blinded throughout the trial, i.e., the study code will not be revealed to the principal investigator or coordinator at anytime during the study.

Patients will be screened to assess whether they meet inclusion/exclusion criteria. Informed consent will then be obtained from the patient. The patient will then be assessed twice per week (at the time compression therapy is administered) for a period of four weeks before randomization occurs. Ulcer surface measurements will be performed at the beginning and at the end of this four week period. Subject whose ulcer surface area has decreased ≥ than 30 % during the four week period prior to randomization. The health nurse will be instructed in the proper care of the ulcer (See Wound Care Section below) as well as in the method of applying DermaLink or the vehicle to the wound.

- The patient will be instructed not to apply ANY other ointments, creams, dressings, or any other treatments to the ulcer during the study.
- The patient will be instructed to raise the foot of the bed that is used for sleeping to form an angle of about 20 degrees, that is to say that he / she will place either a two-inch block under the foot of the bed or two pillows between the base and the mattress of the bed.
- If the patient remains seated for more than 30 minutes after application, he/she will have to raise his/her legs.
- If the patient remains in a fixed standing position after application, he/she must walk every 30 minutes.
- The patient will keep a diary to document these activities.
- The patient diary of activities must be brought in weekly to the clinic for review.

After the initial four-week period, the patient will be randomized to either treatment or vehicle. The above procedure will then be done with the assigned treatment for a period of 12 weeks.

If the patient has more than one ulcer on the same leg, the clinician will target the largest ulcer (between 2-10 cm2) for the study purpose. All of the ulcers on the same leg will be treated the same way (all receiving DermaLink or all receiving vehicle) but only the ulcer targeted will be chosen for analysis of study outcome. Furthermore, if a patient presents ulcers on both legs, the clinician will determine which leg will be chosen for the purpose of the study.

Wound Care

Upon removal of the dressing, inspect wound bed for evidence of infection. If there are signs of infection in the ulcer bed, consult with the clinician for proper care. If there is no evidence of infection then assess the ulcer bed in the following way. If at least 50% of the ulcer bed is pale pink to beefy red (as opposed to gray, yellow, or black indicating necrotic material), then apply the treatment and replacement dressing. If less than 50% of ulcer bed is pink or red, the ulcer bed must be debrided, i.e., cleaning the ulcer of necrotic material. Debridement must be done with surgical sharp instruments and can be performed anytime during the study. Topical anesthesic are allowed.

C ncomitant Medications and/or Procedures

Disallowed medications while on study:

- Chemotherapy
- Prednisone (more than 7.5mg per day)
- Other topical medication on the wound itself including antibiotics

Disallowed treatments/procedures while on study:

- Vascular surgery
- Sclerotherapy

Study Procedures

OBSERVATION PHASE I (4 WEEKS, VISITS 1-8)

All patients will receive the vehicle (calcium-alginate dressing without fibronectin) during this time period. The patient will be seen at the clinic on a twice per week basis.

Screening (day –28, visit 1)

The following information will be obtained and documented on the case report forms:

- Incl. / Excl. Criteria
- Consent form
- Demographic information (age, sex, race, date of birth)
- Serum B-HCG for women of childbearing potential
- FSH for women whom cessation of menses are within 3 years
- Clinical laboratory tests: CBC w/dif, erythrocyte sedimentation rate (ESR), BUN, creatinine, SGOT, SGPT, glucose, albumin, TSH and urinalysis with microscopic examination.
- Serum and plasma samples for fibronectin antibody test and fibronectin plasma level
- Wound culture of normal appearing granulation base
- 3 mm punch biopsy into center of the wound
- Proper surgical debridement of the wound if needed

- Complete medical history. This will include a history on the current ulcer, i.e., which treatments have been used and any adverse effects such as infection. It will also include all previous medical and surgical problems with duration, all known allergies, and cigarette smoking and alcohol consumption history. In addition all concurrent medications will be documented.

- Physical examination to include the following:

- Height, weight, vital signs (pulse, BP, heart rate, oral temperature measured in °C)

- Complete physical exam: general appearance, eyes, ears, nose, throat, thyroid, heart, lungs, abdomen, musculoskeletal system, skin.

- Examination of the arterial vasculature: femoral, posterior tibial, and pedal pulse with gradation from 0 to 3+, presence of livedo, cyanosis, capillary filling time, ankle/brachial index.(ABI)

- Examination of the lower limb: edema, induration, hemosiderosis, Milian's white atrophy, purpura, eczema, dry skin.

- Measurement of calf circumference :

    - mid foot

    - intermalleolar

    - 10 cm above the mid-external malleolus

    - 10 cm below the lower end of the patella

- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, ulcer rim (surrounding skin), sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—mild, moderate, severe), wound discharge and characteristics (color, amount and consistency).

- A tracing of the ulcer will be obtained by placing a grid sheet of transparent acetate over the wound bed and tracing the wound with a fine tip felt marker with permanent ink.

- Drawing of ulcer location on specified CRF.

- Photograph of the ulcer

- Concurrent medication

- Diary given to patient

- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic if judged to be necessary.

- Dressing (vehicle) applied to the wound with high compression therapy using a long stretch four-layer elastic bandage

Observation (visits 2-8)

The following information will be obtained and documented on the case report forms:

- Proper surgical debridement of the wound if needed

- Concurrent medication

- Weight, Vital signs (pulse, BP, heart rate, oral temperature measured in °C)

- Patient's diary review

- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic treatment if judged to be necessary.

- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, ulcer rim (surrounding skin), sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—i.e., mild, moderate, severe), wound discharge and characteristics.

- Dressing (vehicle) applied to the wound with high compression therapy using a long stretch four-layer elastic bandage

Study Treatment (12 weeks, visits 9-33)

The patient will receive either treatment or vehicle during this time period. The patient will be seen at the clinic on a twice per week basis.

Randomisation (day 0, visit 9)

The following information will be obtained and documented on the case report forms:

- Incl. / Excl. criteria

- Urine B-HCG for women of childbearing potential

- Physical exam

- Weight, Vital signs (pulse, BP, heart rate, oral temperature measured in °C)
- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, ulcer rim (surrounding skin), sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—i.e., mild, moderate, severe), wound discharge and characteristics.
- A tracing of the ulcer will be obtained by placing a grid sheet of transparent acetate over the wound bed and tracing the wound with a fine tip felt marker with permanent ink. (ulcers with ≥ 30% reduction in size from *visit 1* will be excluded).
- Photograph of the ulcer
- Examination of the lower limb: edema, induration, hemosiderosis, Milian's white atrophy, purpura, eczema, dry skin.
- Measurement of calf circumference :
    - mid-foot
    - Intermalleolar
    - 10 cm above the mid-external malleolus
    - 10 cm below the lower end of the patella
- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic treatment if judged to be necessary.
- Patient's diary review
- Concurrent medication
- Assessment of wound discharge (ulcers that need more than one change of dressing per week during the last two weeks before randomization (visits 5 to 8) will be excluded.
- Study dressing applied to the wound with high compression therapy using a long stretch four-layer elastic bandage

Treatment (visits 10-33)

The following Information will be obtained and documented on the case report forms:

- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, ulcer rim (surrounding skin), sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—i.e., mild, moderate, severe), wound discharge and characteristics. NOTE: if the wound has healed during this time period, treatment will be discontinued and the patient will be seen once a week for remaining follow up visits to evaluate persistence of closure. Support stocking must be worn until end of study and will be provided by the sponsor.

- Every two weeks, a tracing of the ulcer will be obtained by placing a sheet of transparent acetate over the wound bed and tracing the wound with a fine tip felt marker with permanent ink.

- Concurrent medications

- Weight, Vital signs (pulse, BP, heart rate, oral temperature measured in °C)

- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic treatment if judged to be necessary.

- Patient's diary review

- Study dressing applied to the wound with high compression therapy using a long stretch four-layer bandage

End of study Treatment (day 84, visit 33)

The following information will be obtained and documented in the case report forms:

- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, ulcer rim (surrounding skin), sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—mild, moderate, severe), wound discharge and characteristics.

- A tracing of the ulcer will be obtained by placing a sheet of transparent acetate over the wound bed and tracing the wound with a fine tip felt marker with permanent ink.

- Concurrent medications.

- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic treatment if judged to be necessary..

- Patient's diary review.

- Drawing of ulcer location on specified CRF.
- Photograph of the ulcer.
- Weight, vital signs (pulse, BP, heart rate, oral temperature measured in °C)
- Complete physical exam: general appearance, eyes, ears, nose, throat, thyroid, heart, lungs, abdomen, musculoskeletal system, skin.
- Examination of the arterial vasculature: femoral, posterior tibial, and pedal pulse with gradation from 0 to 3+, presence of livedo, cyanosis, capillary filling time, ankle/arm index.
- Examination of the lower limb: edema, induration, hemosiderosis, Milian's white atrophy, purpura, eczema, dry skin.
- Measurement of calf circumference :
    - mid foot
    - Intermalleolar
    - 10 cm above the mid-external malleolus
    - 10 cm below the lower end of the patella
- Serum and plasma samples for fibronectin antibody test and fibronectin plasma level
- Clinical laboratory tests: CBC w/dif, erythrocyte sedimentation rate (ESR), BUN, creatinine, SGOT,SGPT, glucose, albumin, TSH and urinalysis with microscopic examination.
- High compression therapy using a long stretch four-layer elastic bandage for patients with an unhealed ulcer.
- Support stocking wearing for patients with a healed ulcer. (provided by the sponsor)

Observation phase II (3 months, visits 34-36)

The patient will be seen on a once a month basis.The following information will be obtained and documented on the case report forms:

- Weight, vital signs (pulse, BP, heart rate, oral temperature measured in °C)
- Complete physical exam: general appearance, eyes, ears, nose, throat, thyroid, heart, lungs, abdomen, musculoskeletal system, skin.

- Examination of the arterial vasculature: femoral, posterior tibial, and pedal pulse with gradation from 0 to 3+, presence of livedo, cyanosis, capillary filling time, ankle/arm index.

- Examination of the lower limb: edema, induration, hemosiderosis, Milian's white atrophy, purpura, eczema, dry skin.

- Measurement of calf circumference :

    - mid foot

    - Intermalleolar

    - 10 cm above the mid-external malleolus

    - 10 cm below the lower end of the patella

- Examination of the ulcer with description: ulcer location, presence of necrotic tissue, presence of granulation tissue, presence of fibrinous tissue, crusted surface, sclerosed bottom, redness around the ulcer, swelling around the ulcer, pain (pain characteristics, pain severity—mild, moderate, severe), wound discharge and characteristics.

- A tracing of the ulcer will be obtained by placing a sheet of transparent acetate over the wound bed and tracing the wound with a fine tip felt marker with permanent ink.

- Concurrent medications.

- Assessment for adverse events. Note: if wound infection is suspected, a culture and CBC w/dif must be obtained and patient will be treated with antibiotic treatment if judged to be necessary..

- Drawing of ulcer location on specified CRF.

- Photograph of the ulcer.

- Patient's diary review

- High compression therapy using a long stretch four-layer elastic bandage for patients with an unhealed ulcer.

- Support stocking wearing for patients with a healed ulcer. (provided by the sponsor)

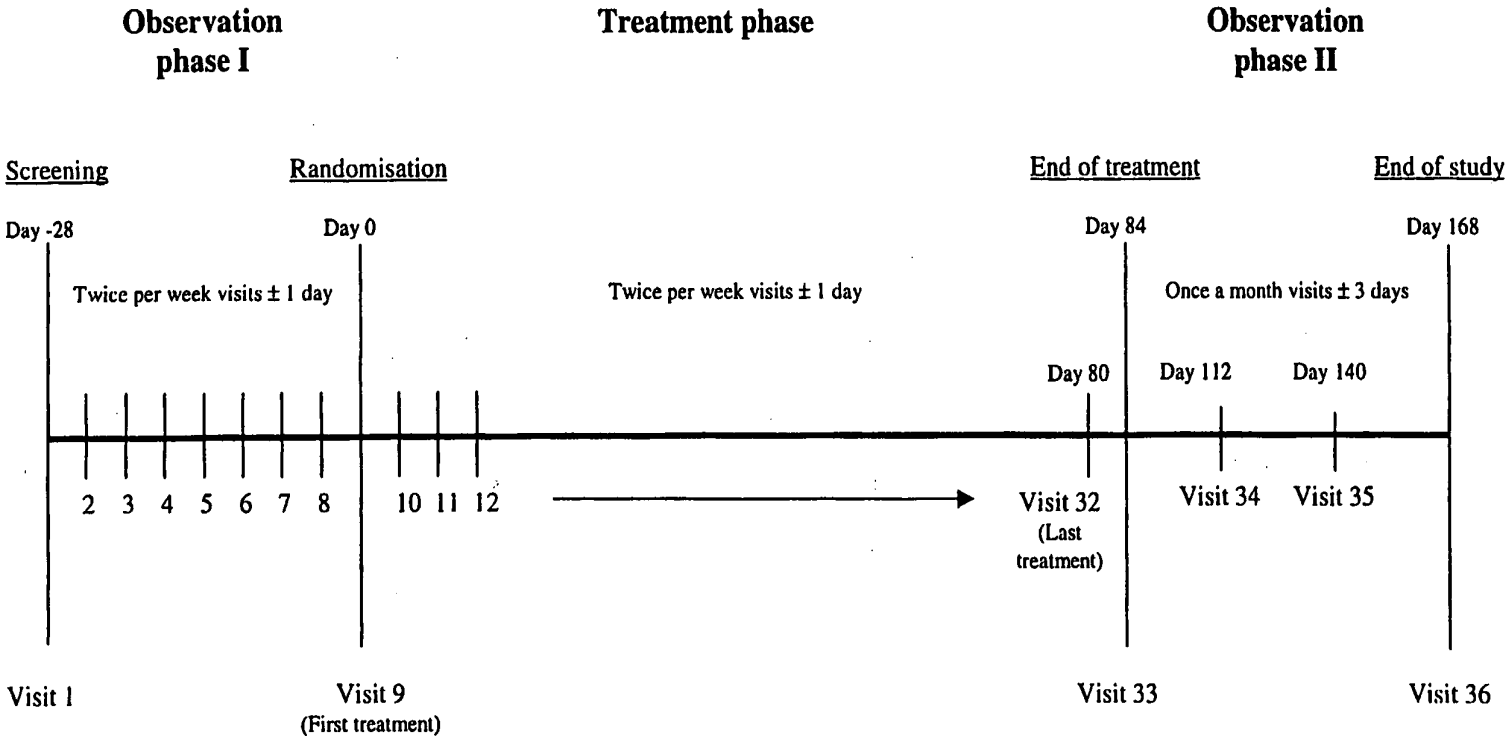
- Serum and plasma samples for fibronectin antibody test and fibronectin plasma level **at visit 36**

Study Flowchart

	Observation phase I		Treatment phase			Observation phase II	
	Visit 1 Screening	Visits 2-8	Visit 9 Randomisation	Visits 10 - 32	Visit 33 End of treatment	Visits 34-36	Premature discontinuation
Incl./Excl. criteria	X		X				
Consent form	X						
Demographic information	X						
Medical history	X						
Physical examination	X		X		X	X	X
Height, weight, vital signs	X		X		X	X	X
Examination of the arterial vasculature	X				X	X	X
Wound culture [a]	X						
3 mm punch biopsy	X						
Proper debridement of wound if needed	X	X	X	X	X	X	X
Concurrent medication	X	X	X	X	X	X	X
Examination of the lower limb [b]	X		X		X	X	X
Measurement of calf circumference	X		X		X	X	X
Examination of ulcer with description [a,c]	X	X	X	X	X	X	X
AcetateTracing of the ulcer	X		X	X (visit 13-17-21-25-29)	X	X	X
Ulcer location (Drawing on theCRF)	X				X	X	X
Photograph of the ulcer	X		X		X	X	X
Serum pregnancy test [d]	X						
Urine pregnancy test [d]			X				
FSH [e]	X						
Clinical lab tests CBC W/Dif, erythrocyte, ESR, BUN, Creatinine, SGOT, SGPT, LDH, glucose, albumin, TSH, urinalysis	X				X		X
Fibronectin Antibody tests (serum) Fibronectin level (plasma)	X				X	X (visit 36)	X
Diary given and reviewed with patient	X	X	X	X	X	X	X
Dressing (vehicle) applied to the wound + high compression therapy	X	X					
Study dressing applied to the wound + high compression therapy			X	X			
Adverse events		X	X	X	X	X	X
End of treatment [f]					X		X

(a) At any time during the study, if wound infection is suspected a culture and CBC W/DIF must be obtained and patient will be treated with antibiotic treatment if judged to be necessary.
(b) Examination of the lower limb. edema, induration, hemosiderosis, Millan's white atrophy, purpura, eczema, dry skin.
(c) Refer to protocol page 8
(d) Serum and urine B-HCG samples for women of childbearing potential only
(e) Women for whom cessation of menses are within 3 years
(f) At any time during the study if the wound has healed, treatment will be discontinued and the patient will be seen once per week for remaining follow-up weeks to evaluate persistence of wound closure.

Timescale


Observation phase I
Treatment phase
Observation phase II
Screening
Randomisation
End of treatment
End of study
Day -28
Day 0
Day 84
Day 168
Twice per week visits ± 1 day
Twice per week visits ± 1 day
Once a month visits ± 3 days
Day 80
Day 112
Day 140
2 3 4 5 6 7 8
10 11 12
Visit 32 (Last treatment)
Visit 34
Visit 35
Visit 1
Visit 9 (First treatment)
Visit 33
Visit 36

MISSED VISITS

If a patient does not keep a scheduled appointment for follow-up, the study site will make very effort to have the patient return as soon as possible.

PREMATURE DISCONTINUATION

Patients have the right to withdraw form the study at any time for any reason. Some common reasons for withdrawal are the patient has an adverse effect and the investigator determines it is in the patient's best interest to discontinue treatment, or if the patient becomes pregnant. If a patient is withdrawn from the study the Investigator will make the best efforts to complete and report the observations. The final study visit (week 16) requirements must be completed at the time of discontinuation. The Investigator will document the reason(s) for withdrawal of each patient in the CRF.

ADVERSE EVENTS

Adverse Events

An adverse event (AE) is an undesirable change in the patient's baseline (either clinical or laboratory) which occurs during the course of a clinical study. The event may or may not be considered as related to the investigational article.

In this study, all adverse events will be recorded in detail on the case report forms, regardless of relationship to the DermaLink. Adverse events such as reaction to study drug or exacerbation of symptoms already existent (like itching, burning sensation, redness, localized oedema at the site), emergence of new symptoms or of a new illness during the study must be recorded.

Serious Adverse Events

A serious adverse event is defined as any adverse experience occurring at any dose that results in the following: death, a life threatening experience, inpatient hospitalization or prolongation of a hospitalization, congenital anomaly or cancer, or a persistent or significant disability/incapacity.

Unexpected Adverse Events

An unexpected adverse event is defined as any experience which is not previously reported (in nature, severity or incidence) in the current Investigator's Brochure, in a previous safety report, in the package insert, in the general investigational plan, or elsewhere.

Reporting of Serious and/or Unexpected Adverse Events

Any serious and/or unexpected adverse event that occurred while on study or 1 month after completion of study must be reported to the sponsor's Clinical Monitor via telephone or e-mail. The Serious Adverse Event Report Form should be completed as soon as the investigator discovers the event. This form should be faxed to the Data Management Center within 5 days of the event. Adverse event reporting will be consistent with the regulatory requirements of Canada and the United States. The Clinical Monitor for this study is listed below.

DR. ?
ADDRESS

CITY
PHONE: ?
E-MAIL: ?

In addition, the local IRB must be notified of all serious adverse events.

Follow up of Adverse Events

The investigator at the site should follow patients with adverse events until the adverse event has resolved or until the condition has stabilized.

Statistical Methods and Data Analysis

The primary efficacy variable in this clinical trial is the time to heal. Secondary effectiveness variables include the proportion of patients in the two study arms who experience complete (100%) closure of their venous ulcer and the proportion of study subjects who experience a successful tissue granulation (determined by serial core biopsy). Complete closure will be determined from the area measurement of the ulcer bed, and the time of closure will be the follow-up visit at which first evidence of closure occurs.

SAMPLE SIZE JUSTIFICATION

A survival analysis of the wound closure can provide a statistical method to test the difference in the rates of healing. The comparison of time to event by use of the log-rank statistic uses information from previous analyses. PASS 6.0 allows the computation of sample size based on such a comparison. The method is based on an approximation developed by Lachin and Foulkes (58). The formula for the overall sample size is given by

$$\sqrt{N}|\lambda_1 - \lambda_2| = Z_\alpha\sqrt{\phi(\bar{\lambda})\left(\frac{1}{p_1}+\frac{1}{p_2}\right)} + Z_\beta\sqrt{\left(\frac{\phi(\lambda_1)}{p_1}+\frac{\phi(\lambda_2)}{p_2}\right)}$$

where p1 and p2 represent the proportion of the total sample size, N, in groups 1 and 2 respectively;

$$\bar{\lambda} = p_1\lambda_1 + p_2\lambda_2$$

represents the average hazard rate for the combined sample; $Z\alpha$ and $Z\beta$ are standard normal variates corresponding to the significance level, α, and statistical power, $1-\beta$; and $\phi(\lambda)$ is a function of the variance of the estimator of the hazard rate, λ. The method assumes an exponential failure time distribution, or alternatively, constant hazard rate for wound closure for each group. The mean λ is the estimate of the common hazard function if the two survival curves are the same, i.e., under the null hypothesis.

The data for the previous study can be used to obtain estimates of the relevant parameters of survival of the ulcer to closure, S1 and S2 for the two groups being compared, because under the exponential assumption the survival is given by

$$S(t) = e^{-\lambda t}$$

and

$$\lambda = -\frac{\log(S(t))}{t}.$$

The null and alternative hypotheses are given below.

$$H_0: S_1 \leq S_2$$

$$H_A: S_1 > S_2$$

Margolis et al. (50) reported that healing of venous leg ulcers is between 30%-60% in 24 weeks with compression therapy which is considered the standard of care. Because of the strict entry criteria and the study observation period of 12 weeks, it is anticipated that the ulcers in this clinical study will have a lower rate of healing because the criteria are designed to screen out the easy to heal wounds. Therefore, the proportion of patients who achieve spontaneous complete wound closure is estimated to be 25%. However, patients with ulcers that are difficult to heal who participate in clinical studies have demonstrated healing rates about 1.5 times higher than those reported in the literature. Thus our estimate of the control healing rate will be 37.5%.

The fibronectin is expected to increase the rate of healing in 12 weeks by about 20%. Thus the treatment group rate of healing in expected to be 57.5%. The number of patients needed to complete the 12 week study period, for a power of 80% to detect a difference in wound survival of between 0.625 (1-0.375) and 0.425 (1-0.575) would be 63 patients in each arm. This sample size accounts for an anticipated 20% dropout rate.

STATISTICAL ANALYSIS

Demographic and Baseline Comparability Analysis - A number of demographic and prognostic variables will be assessed at study entry. These include age, weight, height, and medical history. An analysis of these variables will be done to determine comparability between the fibronectin treated patients and the control patients. For continuous variables such as age or body mass index (BMI), a two-sample t-test or Wilcoxon test will be used. For categorical variables such as prior history of a medical condition or gender, Chi-square or Fisher's exact test will be used. Study site differences may be controlled by analysis of variance or Kruskal-Wallace method for continuous variables and by Cochran-Mantel-Haenszel test for categorical variables. Variables found to be significantly different by treatment group will be included as possible covariates in subsequent safety and effectiveness analyses.

Analysis Populations - The analysis will be done in two ways: intent to treat and as treated. The intent to treat analysis considers the patients in the treatment groups to which they were assigned. The as treated analysis considers patients by the treatment they received. It is possible that a small number of patients may be assigned to one group, but are treated as though they were in the other groups, e.g., a patient is assigned to be a control but the treating clinician mistakenly assigns DermaLink to that patient. In the intent to treat analysis, this patient is included in the control group and in the as treated analysis the patient is included in the DermaLink group.

Primary Efficacy Analysis – The primary efficacy variable is the proportion of patients who achieve completed closure. An analysis of this variable will be assessed at the end of the active treatment phase of the study by Kaplan-Meier survival analysis. To account for possible covariates, these data will also be analyzed by Cox Regression. Primary variables to be considered as possible covariates are size of the ulcer (area), duration of the ulcer, and depth of the ulcer all determined at study randomization. The active arm will be compared to the vehicle control arm. Other variables eligible for the covariate analysis will be those demographic or prognostic variables found to be statistically significant between the groups being compared in addition to variables known or suspected of affecting healing of venous ulcers. This analysis will provide an estimate of the median time to closure by treatment group and will determine if the difference in wound survival between treatment groups is a statistically significant.

Further, a logistic regression (to allow covariate adjustment) will be done to estimate the active treatment effect on the proportion of patients who achieve complete closure in 12 weeks. The same set of covariates used in the Cox regression above will also be applied to the logistic regression. Those variables that are eligible for inclusion in the logistic regression model will be screened for inclusion by their relationship with wound closure (Hosmer and Lemeshow, 59). Categorical variables will be screened by Fisher's exact test or Chi-square. Continuous variables will be screened with a two-sample t-test or Wilcoxon test. All variables with a P-value from the screening test of 0.25 or less will be allowed to enter the model. Variables will be retained in the model if the resulting P-value is 0.10 or less. This analysis method allows the assessment of the role of fibronectin in wound closure adjusted for other factors that also affect wound closure.

Primary Safety Analysis – The adverse events experienced by the treated and control groups will be compared to determine if the frequency, severity, and relatedness of these events are associated with DermaLink treatment. The analysis of choice will depend on the number of events. If the number is small, the proportions experience the total number of adverse events and each individual event will be compared by Fisher's exact test or Chi-square. If the number of events is sufficiently large to warrant a more sophisticated analysis, logistic regression and or Kaplan-Meier analysis will be applied as described above. The safety comparison will combine all patients treated with DermaLink and compare this combined group with the control.

Statistical Testing and Software Information

Superiority analyses will be done with one-sided tests with P-value 0.05. Comparability and safety analyses will be done as two-sided tests with P-value 0.05. The primary analyses will be done with SAS (Version 6.12) for Windows. Supporting and descriptive analyses will be done with StatXact (Version 4.0.1), Systat 8, or Minitab (Version 12.2).

REGULATORY OBLIGATIONS

Informed Consent

The investigators at the sites will obtain written informed consent from each subject participating in this study. The study should be thoroughly explained including the purpose of the study, methods, anticipated benefits and potential hazards prior to obtaining written consent. In addition, subjects must be instructed that they may withdraw their consent at any time during the study. The subjects will also be informed that their medical records may be reviewed by the study monitor, quality assurance auditor, or inspector from the FDA. The subjects will be informed that these persons are bound by the same confidentiality obligations as the patient's physician.

Institutional Review Boards

This protocol will be submitted by the investigator at the site to the appropriate IRB according to local requirements. Approval must be obtained from the local IRB prior to entering any subject on study at that site. In addition, any amendments made to the protocol must be submitted to the local IRB for approval.

Serious adverse events and safety reports must also be reported to the local IRB.

DATA MANAGEMENT

Data Management Center

Data management services will be provided by CL McIntosh & Associates, 12300 Twinbrook Parkway, Rockville, MD, 20852.

Services provided by CL McIntosh for this study include CRF design, database establishment in Oracle ClinicalTM, data collection, review and coding.

Case Report Form Review

Data generated as per protocol will be entered onto the case report forms in accordance with ICH Guideline for Good Clinical Practice. After data is entered onto the case report form, a monitor will compare the data with the source document for verification. Completed case report forms that are monitored will then be sent to the data management center for entry into the database.

Subsequent electronic review of the data may result in queries. These will be sent to the investigator for prompt resolution.

MAINTENANCE OF STUDY SITE FILES

The investigator is responsible for maintaining adequate records to enable the conduct of the study to be fully documented. Copies of the protocol, study approval letters, all CRFs, original patient consent forms, and all correspondence pertaining to the conduct of the study should be kept by the investigator in the study regulatory binder provided by the CRO.

SOURCE DOCUMENTATION

The investigator is responsible for maintaining adequate medical histories and all other pertinent information with regard to the protocol (i.e., laboratory results, recording of measurements, etc.) in the source records for each patient enrolled into the study.

INVESTIGATOR OBLIGATIONS

Monitoring, auditing and inspection

The study monitor will contact and visit the investigator regularly and will be inspecting the various records of the trial. The monitor will be verifying the adherence to the protocol and the completeness, correctness, and accuracy of all CRF entries.

Regulatory requirements

The study will be conducted in compliance with the Helsinki 1989 declaration, local IRB regulations, and Guideline for Good Clinical Practice according to 21 CFR, parts 50, 56, and 312. The Investigator will also maintain patient confidentiality. Patients will only be identified by an identification code consisting of numbers on CRF documents.

CHANGES TO THE PROTOCOL

Protocol amendments

Changes to the protocol may be made by the sponsor in consultation with clinicians, regulatory consultants and statisticians out of necessity. All substantive changes will be submitted to the FDA and the clinical site's IRB for approval. Amended protocols and copies of IRB approval letters will be kept on file at the site in the regulatory binders.

STUDY TERMINATION

The study may be terminated at any time by the sponsor or FDA. The sponsor and investigator will ensure that adequate consideration is given to the protection of the patients' interest.

STUDY REPORT

A report of the clinical study will be prepared by the sponsor after the completion of the study and submitted to the investigators for review. The report will be incorporated into a regulatory submission intended to get market clearance for the product.

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Alginates

Alginates are naturally occuring substances extracted from marine brown algae and are used in the pharmaceutical, cosmetic, textile and food industry. Alginates are polyanionic polysaccharides composed of linear binary copolymers of D-mannuronic acid and L-guluronic acid. The most common uses are based on the polyelectrolytic nature of the alginates, which forms the basis gelling properties and their ability to swell. The commercially available sodium alginates are water soluble. When adding such alginates to a solution containing polyvalent ions, for example bivalent alkaline earth metal ions such as Ca, alginate gels having a defined form are produced. This is a result of a ionic crosslinking of several alginate chains.

Calcium alginate dressings

Calcium alginates have long been known for their ability to form fibres or nonwoven materials primarily for use as swabs or dressings for medical, surgical or other purposes. Supplied in the form of nonwoven wound dressings for the treatment of exudating wounds, the calcium alginate dressing is said to encourage the formation of controlled ion-active gel over the wound site wich reacts with the sodium ions in exudate. Calcium alginates dressings in particular are recommended for use on exudating wounds, such as pressure ulcers, venous stasis ulcers, diabetic ulcers, arterial ulcers, second degree burns and skin graft donor sites.

Solid fibronectin calcium-alginate wound dressing formulation

Alginate salts can be converted into fibers by a process of freeze-drying. This procedure produces a sponge like structure with hydrophilic properties. In the presence of fluids, the dressing turns into a gel-like state, capable of

absorbing up to 20 times its weight in wound exudate. The fibrous gel creates the desired moist environment for the wound. The dressing can be removed with a minimal amount of discomfort and granulating tissue and epithelial cells are not traumatized during dressing change.

By combining the beneficial effect of calcium alginate and fibronectin, a calcium alginate dressing was developed with the ability to deliver a high concentration of fibronectin into the wound site. The basic mechanisms at play are that when the fibronectin-calcium alginate dressing comes into contact with the sodium in the exudate, ion exchange occurs, turning the calcium alginate fibers into a protective non-adherent film gel. In this gel state, fibronectin is free to move from the gel into the wound.

Solubilization

$$Ca\ (alginate)_2 + 2\ Na^+ \text{ ----------> } 2\ Na\ (alginate) + Ca^{++}$$

Fibronectin-calcium alginate dressing composition

The fibronectin-calcium alginate wound dressing is prepared as follows: 5 g of sodium alginate (Protanal LF 10/60, Pronova Biopolymer, Drammen, Norway) are dissolved in 95 g of deionized and demineralized water with a paddle type stirrer for about 1 hour. This dispersion provides a concentrated alginate base (5 % w/w). The pH of 10 mL demineralized water is adjusted at pH 8.0 to 11.0 with the addtion of 3.35 µg to 3.35 mg NaOH 3M. The lyophilized fibronectin is next dissolved in demineralized water pH 8.0 to 11.0 in quantities varying from 0.025 to 0.1 g. The solution is maintained at 37°C until complete solubilization of fibronectin occurs and is then filtered through a 0.22 µm acetate filter. The 1% sterile solution of fibronectin (3.4 mL) is then mixed into syringes with a sterile filtered 1% solution sodium alginate (1.6 mL) in mild acetic acid pH 4.0 (3.3 µL of glacial acetic acid 17.4N in 100 Ml of demineralized water gives a pH of 4.0) prepared from the concentrated alginate base. Gellation of the solution is achieved by the addition of 15 µL

0.2 M NaCl + 0.2 M $CaCl_2$ and 3.4 µL of glacial acetic acid. At this point, the fibronectin-calcium alginate complex is deposited in a borosilicate glass vial (5 mL for a surface area of 5.3 cm^2) and frozen at -20°C for 2 hours and 30 minutes at -80°C. The water is then removed by freeze-drying. By this technique, a 4.2 cm^2 solid sponge-like fibronectin-calcium alginate wound dressing with a fibronectin concentration up to 80 µg/mm^2 is produced.

Discussion

According to a first aspect of the present dressing, the wound sponge-like dressing, comprises a mixed salt alginate which has a first and second cations, the first cation (Ca^{2+}) being capable of forming an insoluble alginate salt and the second cation (Na^+) being capable of forming a soluble alginate salt. The equivalent ratio of the first to second cations being 50:50 (here 0.2M NaCl and 0.2M $CaCl_2$). The maximum homogeneity in the dressing is reached by a concentration of both gelling and non-gelling agents.

Because alginates are anionic polysaccharides, the complex is preferably formed by combining the fibronectin and sodium alginate at a pH which is no higher than the isoelectric point of the protein (pI 6.2), where the fibronectin is positively charged. This is achieved in a final step by adding glacial acetic acid for a final pH around 5.0. Because acetic acid is highly volatil, during the freeze-drying process which is under vacuum, a certain amount of acetic acid is removed. The final pH of the dressing is then around 6.5.

It has been found that the mixed salt alginate dressing exibits a highly effective combination of properties. There is sufficient insolubilising cation in the mixed salt alginate to be relatively easy to be manipulated and there is sufficient solubilising cation allowing the release of fibronectin to the wound

site. The release of fibronectin is also achieved by wetting the dressing with saline solution. In the rabbit animal model, 40 µl of saline solution (Aqualite® 0.9% sodium chloride solution) is used to wet a calcium-alginate dressing produced using a 9mm AcuPunch® biopsy punch.

The combination of soluble and insoluble alginate fibres has the further advantage that the dressing is easily removed after wound treatment.

It should be noted that solid wound dressings could also be prepared with other insolubles fibres. They could be any insoluble fibres or materials which does not have adverse effect on the wound. Examples of suitable plant polysaccharides are carrageenans and cellulose derivatives for instance carboxymethylcellulose or hydroxypropylcellulose. A synthetic carbomer resin could also be used.

Composition of different solid wound dressings

Solid Carboxymethylcellulose (CMC) dressing

Solid carboxymethylcellulose (CMC) dressing was prepared. Preferred grade is GPR® (BDH Laboratories, Ville St-Laurent, Canada). A solid wound dressing containing (w/w) fibronectin 62%, CMC 38% was prepared as follows. CMC powder was first sterilized by using a dry-heat sterilization process. CMC (6g) was dispersed in 94 mL of deionized water and allowed to be mixed with a paddle type stirrer for about 3 hours. This provides a sterile concentrated hydrogel base (6% w/w). Lyophilized fibronectin (50 mg) was dissolved in deionized water (5 mL) containing 12 µL of NaOH 3M, pH 11.6. The solution was maintained at 37°C until complete solubilization of fibronectin occured. This stock solution of fibronectin 10 mg/mL was filtered through a 0.22 µm acetate filter. Fibronectin solution (3.3 mL) was then

added to a portion (0.34 g) of concentrated CMC base and mixed into syringes. The pH is adjusted at 7.0 with the addition of 25 µL HCl 1 N. At this point, the homogenous solution of the fibronectin-CMC complex is deposited in a plastic mold and frozen. The water is then removed by freeze-drying. By this technique, a fibronectin-CMC wound dressing with a sponge-like structure is produced.

Solid Hydroxypropylcellulose (HPC) dressing

Solid hydroxypropylcellulose (HPC) dressing was prepared. Preferred grade is Klucel-HF® (Aqualon, Houston, Texas). A solid wound dressing containing (w/w) fibronectin 45%, HPC 55% was prepared as follows. HPC powder was first sterilized by using a dry-heat sterilization process. HPC (6 g) was dispersed in 94 mL of deionized water and allowed to be mixed with a paddle type stirrer for about 3 hours. This provides a sterile concentrated hydrogel base (6% w/w). Lyophilized fibronectin (50 mg) was dissolved in deionized water (5 mL) containing 12 µL of NaOH 3M, pH 11.6. The solution was maintained at 37°C until complete solubilization of fibronectin occured. This stock solution of fibronectin 10 mg/mL was filtered through a 0.22 µm acetate filter. Fibronectin solution (3.3 mL) was then added to a portion (0.68 g) of concentrated HPC base and mixed into syringes. The pH is adjusted at 7.0 with the addition of 25 µL HCl 1 N. At this point, the homogenous solution of the fibronectin-HPC complex is deposited in a plastic mold and frozen. The water is then removed by freeze-drying. By this technique, a fibronectin-HPC wound dressing with a sponge-like structure is produced.

Solid carbomer dressing

Solid carbomer dressing was prepared. Preferred grade is Carbopol® 974P NF (BF Goodrich, Cleveland, Ohio). A solid wound dressing containing (w/w)

fibronectin 75%, carbomer 25% was prepared as follows. Carbomer (2.80 g) was dispersed in 97.2 mL of deionized water and allowed to be mixed with a paddle type stirrer for about 3 hours. This dispersion is then autoclaved to provide a sterile concentrated hydrogel base (2.80% w/w). Lyophilized fibronectin (50 mg) was dissolved in deionized water (5 mL) containing 12 µL of NaOH 3M, pH 11.6. The solution was maintained at 37°C until complete solubilization of fibronectin occured. This stock solution of fibronectin 10 mg/mL was filtered through a 0.22 µm acetate filter. Fibronectin solution (3.3 mL) was then added to a portion (0.04 g) of concentrated carbomer base and the necessary amount of gelifying promoter (25 µL NaOH 3M) and mixed with syringes. This fibronectin carbomer hydrogel is deposited in a plastic mold and frozen. The water is then removed by freeze-drying. By this technique, a fibronectin-carbomer wound dressing with a sponge-like structure is produced.

Solid carrageenan dressing

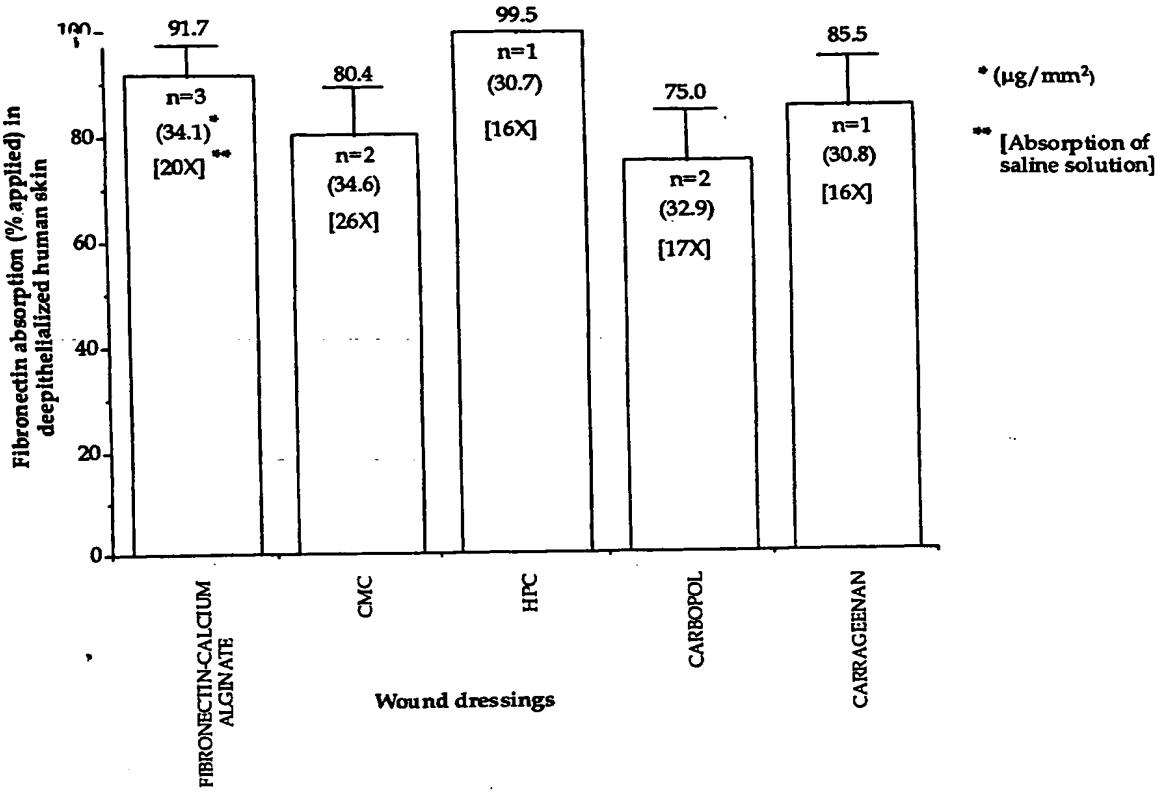
Solid carrageenan dressing was prepared. Preferred grade is Gelcarin® NF (FMC Corporation Pharmaceutical Division, Newark, Delaware). A solid wound dressing containing (w/w) fibronectin 73%, carbomer 27% was prepared as follows. Carrageenan (2.50 g) was dispersed in 97.5 mL of deionized water and allowed to be mixed with a paddle type stirrer for about 3 hours. This dispersion is then autoclaved to provide a sterile concentrated hydrogel base (2.50% w/w). Lyophilized fibronectin (50 mg) was dissolved in deionized water (5 mL) containing 12 µL of NaOH 3M, pH 11.6. The solution was maintained at 37°C until complete solubilization of fibronectin occured. This stock solution of fibronectin 10 mg/mL was filtered through a 0.22 µm acetate filter. Fibronectin solution (3.3 mL) was then added to a portion (0.50 g) of concentrated carrageenan base and mixed into syringes. The pH is adjusted at 7.0 with the addition of 60 µL HCl 1 N. At this point, the

We claim:

1. A pharmaceutical delivery system comprising a calcium-alginate dressing containing an effective amount of a wound healing promoter.

2. A pharmaceutical delivery system according to claim 1, wherein the wound healing promoter is fibronectin.

3. A pharmaceutical delivery system according to claim 2 with a fibronectin concentration up to $80\mu g/mm^2$.



Absorption of fibronectin in deepithelialized human skin using different solid wound dressings. The number in ( ) refers to the quantity of absorbed fibronectin (µg) per mm^2 of deepithelialized human skin over a 12 hour period. The number in [ ] refers to the quantity of absorbed saline solution (0.9% NaCl) by weight of dressing. Bars represent standard deviations of the mean.

Absorption of fibronectin in deepithelialized human skin using different solid wound dressings over a 24 hour period

FIG 1